Clinical Study Protocol: KTX-101

Study Title:	A Phase 1 Study Investigating the Safety, Tolerability and Pharmacokinetics of KNX100 in Healthy Volunteers
Study Number:	KTX-101
Study Phase:	1
Product Name:	KNX100 (active ingredient: KNX100 Phosphate)
IND Number:	IND 155,839
Clinical Trials.Gov Number:	NCT04901078
Indication:	Mitigation of opioid withdrawal symptoms
Investigators:	Single center (Australia)
Sponsor:	Kinoxis Therapeutics Pty, Ltd (Kinoxis) Suite 201, 697 Burke Road Camberwell Victoria 3124 Australia
Sponsor Contact:	Dr. Tina Soulis Vice President, Clinical Strategy and Development Kinoxis Therapeutics Pty Ltd. tina.soulis@kinoxistherapeutics.com

Amendment	Version	Date
Original Protocol:	1.0	21 September 2021
Amendment 1	2.0	05 January 2022
Amendment 2	2.1	12 May 2022
Amendment 3	3.0	29 September 2022
Amendment 4	4.0	29 December 2022
Amendment 5	5.0	20 February 2023
Amendment 6	**6.0**	**07 July 2023**

Confidentiality Statement

SPONSOR SIGNATURES

Study Title:	A Phase 1 Study Investigating the Safety, Tolerability and Pharmacokinetics of KNX100 in Healthy Volunteers
Study Number:	KTX-101
Final Date:	07 July 2023

This study clinical study protocol was subjected to critical review and the information it contains is consistent with the moral, ethical, and scientific principles governing clinical research as set out in the Declaration of Helsinki and the Principles of Good Clinical Practice (GCP) as described in the International Council for Harmonisation (ICH) E6 GCP guideline and Title 21 Code of Federal Regulations parts 50, 54, 56, and 312.

This protocol has been approved by the Sponsor for execution. The following personnel contributed to writing and/or approving this protocol:

Signed: Sharon Hanegraaf Date: Jul 7, 2023

Sharon Hanegraaf
VP, Drug Development
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Signed: Date: Jul 7, 2023

Tina Soulis, Ph.D.
VP, Clinical Strategy and Development
Kinoxis Therapeutics Pty Ltd

INVESTIGATOR'S SIGNATURE

Study Title:	A Phase 1 Study Investigating the Safety, Tolerability and Pharmacokinetics of KNX100 in Healthy Volunteers
Study Number:	KTX-101
Final Date:	07 July 2023

I have read the KTX-101 protocol and agree to conduct the study as outlined.

I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

I agree to conduct the study in accordance with the ethical principles that have their origin in the Declaration of Helsinki, are consistent with the Good Clinical Practices guidelines of the International Council for Harmonisation, and according to applicable regulatory requirements.

Principal Investigator Signature: _____

Print Name: _____

Print Title: _____

Institution/Affiliation: _____

Date of Signature: _____

SYNOPSIS

Study Title: A Phase 1 Study Investigating the Safety, Tolerability and Pharmacokinetics of KNX100 in Healthy Volunteers	
Name of Finished Product: KNX100	**Name of Active Ingredient:** KNX100 Phosphate
Study Number: KTX-101	**Study Phase:** 1
Study Centers: One site (Australia)	
Primary Objectives: The primary objectives of this study are to evaluate the safety and tolerability of KNX100 administered orally as a single ascending dose and multiple ascending doses in healthy volunteers.	
Secondary Objectives: The secondary objective of this study is to determine the pharmacokinetic (PK) profile of KNX100 and its metabolites following administration of KNX100 as single and multiple oral doses in healthy volunteers.	
Study Design: This is an adaptive, Phase 1, first-in-human (FIH), single treatment, double blind, placebo controlled, randomized, single/multiple ascending dose study of KNX100 administered to healthy volunteers (Part A and B). Approximately 64 male and female healthy subjects will be enrolled into Part A and B in this study. Healthy subjects who meet all the eligibility criteria will be randomly assigned to Cohorts 1-4 in Parts A or Cohorts 1-3 in Part B of the study. Each cohort will evaluate 8 subjects; 6 subjects will receive KNX100 (study drug), and 2 subjects will receive placebo. Additional cohorts may be added as required, based on safety profile and Cohort Review Committee (CRC) authorization. Each cohort will be enrolled sequentially, and dose escalation decisions will be made according to protocol by the CRC consisting of the investigators and medical monitor. Subjects and clinical staff will be blinded to therapy assignment.	
Planned Number of Patients: Approximately 64 healthy subjects will be randomized for study participation. The total number of subjects will depend on the number of dose levels assessed during dose-escalation to determine the Recommended Phase 2 dose (RP2D) of KNX100. Randomization will be controlled either via a list or Interactive Web Response System.	
Diagnosis and Main Criteria for Inclusion: This study will evaluate healthy volunteers (male and female) who are between 18 and 55 years of age (inclusive) with a body mass index (BMI) of 18-32 (inclusive). Subjects must be willing to comply with study requirements and must agree to abstain from consuming alcohol or using recreational drugs. Subjects with specific underlying conditions (e.g., mental illness, human immunodeficiency virus (HIV), hepatitis, cardiovascular disease) or any medical history that would jeopardize their safety cannot participate. Additionally, subjects with clinically significant electrocardiogram (ECG),	

electroencephalogram (EEG), physical examination, or laboratory findings will not be enrolled. Female subjects of childbearing potential must be willing and able to use defined methods of contraception throughout the study and for 30 days after the final follow-up visit.
Test Product; Dose; and Mode of Administration: KNX100 will be provided in capsule form as 5, 25 or 100 mg capsules for oral administration. Study drug will be encapsulated in hydroxypropyl methylcellulose (HPMC) dark green opaque size 0 capsules and packaged in 100 mL high density polyethylene (HDPE) bottles with polypropylene twist-off closures. Dosing will be based on the assigned treatment group. This study will evaluate doses of KNX100 starting with a single ascending dose (Part A, SAD) of 5 mg, increasing up to a maximum of 50 mg and a multiple ascending dose (Part B, MAD) starting at 10 mg and increasing up to 35 mg per dose, as tolerated. Individual doses will be dispensed by unblinded site pharmacy staff. Dose escalation will progress upon CRC approval. Subjects will take the study drug in the morning after an overnight fast. For subjects enrolled into MAD Cohort 3, subjects will take study drug in the morning after an overnight fast and again, approximately 8 hours after the first dose following a 2-hour fast.
Reference Therapy; Dose; and Mode of Administration: KNX100 matching placebo will be provided in capsule form for oral administration. The placebo will be encapsulated in HPMC dark green opaque size 0 capsules and packaged in 100 mL HDPE bottles with polypropylene twist-off closures. Dosing will be based on the assigned treatment group. An equivalent number of capsules will be administered to subjects receiving placebo (e.g., the first dose of study drug will be one 5 mg capsule; subjects receiving placebo will also receive only one capsule). Individual doses will be dispensed by unblinded site pharmacy staff. Subjects will take the study drug in the morning after an overnight fast. For subjects enrolled into MAD Cohort 3, subjects will take study drug in the morning after an overnight fast and again, approximately 8 hours after the first dose following a 2-hour fast.
Duration of Treatment: The length of the treatment period for each subject in Part A is 1 day (1 dose administered). For Part B Cohorts 1 and 2, the length of the treatment period for each subject is 7 days (7 doses administered). For Cohort 3, the length of the treatment period for each subject is 7 days (14 doses administered). During Part A, subjects will remain in the Phase 1 unit for 30 hours after study drug administration for PK sampling and safety evaluations. For Part B, subjects will remain in the Phase 1 unit for 7 days of study drug administration, PK sampling and safety evaluations, and be discharged on Day 8 after PK and safety evaluations.

Duration of Patient Study Participation:

Subjects in Part A (SAD) will be on study for up to 38 days:

- Screening: up to 28 days
- Treatment: 1 day
- Follow-up: 7 (± 2) days following completion of treatment

Subjects in Part B (MAD) will be on study for up to 44 days:

- Screening: up to 28 days
- Treatment: 1 dose each day for 7 days (Cohorts 1 and 2)
  1 dose administered twice daily for 7 days (Cohort 3)
- Follow-up: 7 (±2) days following completion of treatment

Safety Assessments:

The primary safety endpoints include:

- Incidence of treatment emergent adverse events (TEAEs), treatment emergent serious adverse events (TESAEs), related adverse events (AEs), AEs leading to discontinuation, and AEs by severity.
- Clinical laboratory testing from protocol-specified standard urine and blood tests, including liver function tests (LFTs) and thyroid function tests (TFTs: triiodothyronine [T3], thyroxine [T4], thyroid stimulating hormone [TSH]).
- Change in clinical observations from baseline, including body temperatures, vital signs, ECGs, and EEGs.

Pharmacokinetic Variables:

The secondary PK endpoints include assessment of:

- Maximum observed concentrations (C_{max}) following dose administration.
- Time of C_{max} (T_{max}) following dose administration.
- Area under the concentration-time curve (AUC) from time zero to last quantifiable concentration ($AUC_{0\text{-}last}$), from time zero to 24 hours postdose administration ($AUC_{0\text{-}24}$) and, as data permit, from time zero to extrapolated to infinity ($AUC_{0\text{-}\infty}$).
- As data permit, additional disposition parameters including terminal half-life ($t_{1/2}$), volume of distribution (V_z/F), and oral clearance (CL/F) may be calculated.
- Accumulation ratio (RAC) for C_{max} and $AUC_{0\text{-}24}$; Day 7 vs Day 1 (MAD phase only).
- Other PK parameters will be calculated as deemed appropriate.

Statistical Methods: All data will be presented in the data listing. AEs will be summarized for each treatment and cohort and overall, by system organ class and preferred term to include the number and percentage of subjects who experience at least one AE. AEs will also be summarized by relatedness, severity, study drug discontinuation, and seriousness. Clinical laboratory results, vital signs, body temperature, pulse oximetry measurements, EEGs and

single-lead ECG results will be summarized by actual values and change from baseline. Physical examination findings will be presented in a data listing.

Plasma concentration data will be summarized over time by treatment using descriptive statistic (number of observations, arithmetic mean, standard deviation, arithmetic coefficient of variation [CV], geometric mean, geometric CV, median, minimum, and maximum). Mean and individual plasma concentration versus time profiles will be presented in figures on both linear and semi logarithmic scales.

For the PK parameters, descriptive statistics will be provided for each parameter (number of observations, arithmetic mean, standard deviation, arithmetic CV, geometric mean, geometric CV, median, minimum, and maximum).

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LIST OF ABBREVIATIONS AND DEFINITIONS

Abbreviation	**Definition**
ADL	activities of daily living
AE	adverse event
ALT	alanine aminotransferase
AUC_{0-t}	area under the concentration-time curve from time zero to last quantifiable concentration
$AUC_{0-\infty}$	area under the concentration-time curve from time zero to infinity
AUC_{0-24}	area under the concentration-time curve from time zero t0 24 hours
b.i.d	twice daily
BLQ	below the limit of quantification
BMI	body mass index
CDC	Centers for Disease Control
CFR	Code of Federal Regulations
CL	clearance
CL/F	oral clearance
C_{max}	maximum plasma drug concentration
C_{min}	minimum plasma drug concentration
CNS	central nervous system
CRA	clinical research associate
CRC	Cohort Review Committee

Abbreviation	**Definition**
CRO	contract research organization
CSSRS	Columbia Suicide Severity Rating Scale
CTCAE	Common Terminology Criteria for Adverse Events
CV	coefficient of variation
DMP	data management plan
DSM-5	Diagnostic and Statistical Manual of Mental Disorders
eCRF	electronic case report form
ECG	electrocardiogram
EEG	electroencephalogram
EOS	end of study
ET	early termination
FDA	Food and Drug Administration (US Government)
FIH	first-in-human
FSH	follicle stimulating hormone
GCP	Good Clinical Practice
HDPE	high density polyethylene
HED	human equivalent dose
HIV	human immunodeficiency virus
HPMC	hydroxypropyl methylcellulose
HREC	Human Research Ethics Committee (Australian equivalent of IRB)
ICH	International Conference on Harmonisation
IED	interictal epileptiform discharges
IND	Investigational New Drug
IP	intraperitoneal
IUD	intrauterine device
KNX100	Study drug: 1-methyl-1,4,5,10-tetrahydropyrazolo[3,4-b][1,5]benzodiazepine phosphate
LFT	liver function test
LOEL	lowest-observed-effect-level
MedDRA	Medical Dictionary for Regulatory Activities
MAD	multiple ascending dose

Abbreviation	Definition
MAT	medication-assisted treatment
MRSD	maximum recommended starting dose
MRT	mean residence time
MTD	maximum tolerated dose
NAcSh	nucleus accumbens shell
NHMRC	National Health and Medical Research Council (Australian government)
NOAEL	no-observed-adverse-effect level
NOEL	no-observed- effect-level
OTC	over-the-counter
OUD	opioid use disorder
PAD	pharmacologically effective dose range
PD	pharmacodynamics
PK	pharmacokinetics
PI	principal investigator
PP	per protocol set
RAC	accumulation ratio
RP2D	recommended phase 2 dose
SAD	single ascending dose
SAE	serious adverse event
SAF	Safety Set
SAR	suspected adverse reaction
SID	Subject Identification Number
SD	Standard deviation
SOA	Schedule of Assessments
SSI	significant safety issue
SUSAR	suspected unexpected serious adverse reaction
$t_{1/2}$	half life
T3	free triiodothyronine
T4	thyroxine
TEAEs	treatment emergent adverse events
TESAEs	treatment emergent serious adverse events

Abbreviation	**Definition**
TFT	thyroid function test
TGA	Therapeutic Goods Administration
T_{max}	time to maximum plasma concentration
TSH	thyroid stimulating hormone
Vz/F	apparent volume of distribution
US	United States
USM	urgent safety measures
WOCBP	women of childbearing potential

1. INTRODUCTION

1.1. Disease Background

1.1.1. Opioid Use Disorder

Opioid use disorder (OUD) is defined in the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) as a problematic pattern of opioid use leading to clinically significant impairment or distress. The DSM-5 diagnostic criteria for OUD include presentation of at least 2 of the following:

- Opioids are often taken in larger amounts or over a longer period than was intended.
- There is a persistent desire or unsuccessful efforts to cut down or control opioid use.
- A great deal of time is spent in activities necessary to obtain the opioid, use the opioid, or recover from its effects.
- Craving, or a strong desire or urge to use opioids.
- Recurrent opioid use resulting in a failure to fulfill major role obligations at work, school, or home.
- Continued opioid use despite having persistent or recurrent social or interpersonal problems caused or exacerbated by the effects of opioids.
- Important social, occupational, or recreational activities are given up or reduced because of opioid use.
- Recurrent opioid use in situations in which it is physically hazardous.
- Continued opioid use despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance.
- Exhibits tolerance.
- Exhibits withdrawal.

Opioids, a broad class of drugs that act on opioid receptors in the brain, spinal cord, and other parts of the body are commonly prescribed for pain, with approximately 3%-4% of the adult US population prescribed long-term opioid therapy (Boudreau et al., 2009; Volkow and McLellan 2016), however, long-term use can be associated with misuse, addiction, morbidity, and mortality. The opioid (opioid analgesics/heroin/fentanyl) overdose epidemic is among the most pressing public health issues in the US (Volkow et al., 2014). Opioid overdose is the number one cause of preventable deaths in the United States (US), killing more people than motor vehicle accidents. In Australia, opioids accounted for just over 3 deaths a day in 2018 and were linked to more than half of the drug induced deaths across the country. Poor management of opioid withdrawal is a major contributor to the development and maintenance of harmful opioid use. Opioid-associated morbidity and mortality are described by the Centers for Disease Control (CDC) as a national epidemic which continues to escalate, with a record 68,821 opioid overdose

deaths recorded in 2020 (CDC WONDER). In 2020, over 92,000 people died from drug overdoses, making it a leading cause of injury-related death in the US and of those deaths, over 70 percent involved a prescription or illicit opioid (CDC 2021). These alarming trends led the US Department of Health and Human Services (HHS) to deem prescription-opioid overdose deaths an epidemic and prompted multiple federal, state, and local actions (CDC 2013).

1.1.2. Opioid Withdrawal

Opioid withdrawal is the rapid onset of severe physical and mental distress following a partial or total reduction of opioid consumption. Portenoy (1996) observed that addiction occurred due to 2 factors: the inherent reinforcing properties of the opioid drugs, and the psychological/social/ physiological factors of the individual which predispose to addiction. Abrupt cessation of opioids in physically dependent subjects results in acute withdrawal symptoms. The time of onset, peak, and duration of these symptoms depends on the elimination half-life of the opioid that was used prior to cessation, but the symptoms are essentially the same irrespective of the specific opioid.

Opioid withdrawal symptoms develop within several hours to a few days following termination or reduction of heavy or prolonged opioid use. Symptoms include sweating, rapid pulse, increased hand tremor, insomnia, nausea, vomiting, physical agitation, anxiety, transient visual, tactile, or auditory hallucinations/illusions and grand mal seizures (Dijkstra et al., 2007). Opioid withdrawal symptoms occur both in subjects who have been using opioids appropriately, and in subjects with OUD. In subjects with OUD, the occurrence of withdrawal symptoms and the relief of withdrawal when opioids are used are important drivers of continuing use.

1.1.3. Currently Available Treatments for Opioid Withdrawal

Medication-assisted treatment (MAT) is the primary treatment for OUD. However, only a limited number of FDA approved pharmacological treatment options are currently available for OUD and they are underutilized: fewer than 20% of persons in the US with OUD receive an OUD-specific treatment (Wu et al., 2016). Drugs approved to treat OUD are presented in Table 1, along with their specific FDA approved indication(s).

Table 1 FDA Approved Medications for Opioid Use Disorder Indications

Medication	Mechanism	FDA Label Indication (Abbreviated)
Naloxone	Opioid receptor antagonist	Opioid overdose
Lofexidine	Adrenergic receptor agonist	Opioid withdrawal
Methadone	Opioid receptor agonist	Detoxification, maintenance treatment
Buprenorphine	μ-opioid receptor partial agonist	Detoxification, maintenance treatment
Buprenorphine/naloxone	See above	Maintenance treatment
Naltrexone	Opioid receptor antagonist	Relapse prevention following detoxification

The drug treatments presented in Table 1 suffer from a mixture of suboptimal efficacy and safety which contributes to poor uptake and treatment adherence. For example, lofexidine, the first FDA approved drug for mitigating opioid withdrawal symptoms following abrupt opioid discontinuation, achieved only modest improvements in acute opioid withdrawal symptoms and treatment retention, while causing concerning side-effects, including hypotension, bradycardia, and insomnia (Gorodetzky et al., 2017; Choy, 2018). Naltrexone and maintenance treatments suffer from both poor treatment initiation and compliance, with a recent large cohort study finding only 11%, 17%, and 6% of subjects initiated methadone, buprenorphine, or naltrexone treatment respectively, within 12 months of a nonfatal overdose, with median treatment retention periods of just 5, 4, and 1 month(s) (Larochelle et al., 2018).

Despite the limited number of treatment options currently available and their underutilization, there is very little in the clinical pipeline for the treatment of OUD. A recent pharmaceutical industry report identified only 29 current clinical programs for substance use disorders. Of these, only 15 were ongoing clinical programs testing new molecular entities (NMEs), with no novel clinical programs specifically targeting OUD (Thomas and Wessel, 2018). Clearly there is an urgent need to develop novel pharmacological treatments for OUD and, importantly, to progress novel treatments expeditiously into clinical development.

1.2. Rationale for KNX100

Kinoxis Therapeutics Pty Ltd (Kinoxis) is developing a new chemical entity, KNX100 as a small molecule intended for the treatment of symptoms associated with opioid withdrawal in patients with OUD. KNX100 was originally developed by the University of Sydney, Australia and is now owned by Kinoxis. KNX100 was selected from a number of molecules under development as it demonstrated potent anti-addictive and prosocial effects in several different animal models and activated brain regions and cell types of interest.

The opioid crisis in the US continues to escalate, with a record 49,860 opioid overdose deaths recorded in 2019 (CDC WONDER). Treating OUD, including managing opioid withdrawal symptoms, presents a considerable challenge for health care professionals and there are currently only a limited number of drugs approved for the treatment of OUD (including treatments for overdose and withdrawal). These include μ-opioid receptor antagonists (naloxone and naltrexone), opioid maintenance treatments (μ-opioid receptor agonist and partial agonists, methadone and buprenorphine, respectively, and combination partial agonist/antagonist treatments), and the α_2-adrenergic agonist, lofexidine. Opioid maintenance therapies are associated with risk of overdose, diversion, abuse, and compliance issues. Naloxone is primarily used to treat overdose and naltrexone suffers from uptake, compliance, and efficacy issues. Lofexidine demonstrates limited efficacy and has significant cardiovascular liabilities.

Pharmacologically, opioids bind to opioid receptors located in the brain, spinal cord, and other parts of the body. Activation of opioid receptors in different parts of the central and peripheral nervous systems mediates the various effects of opioids. These include analgesia, respiratory depression, and opioids' addictive properties. Opioid withdrawal occurs following a discontinuation or reduction of opioid use, or administration of a μ-opioid receptor partial agonist or antagonist. Opioid withdrawal syndrome consists of often severe somatic (e.g., hyperalgesia, gastrointestinal upset, and chills) and affective (e.g., anxiety, dysphoria, and intense craving for opioids) symptoms. Withdrawal typically begins within 1-2 days of the last dose of an opioid or commencement of dose tapering, and symptoms can last for 2-4 weeks or longer. Opioid withdrawal is caused by adaptations that occur in the nervous system as a result of repeated activation of opioid receptors. Poor management of opioid withdrawal is a major contributor to the development and maintenance of harmful opioid use as it is the first barrier to switching to maintenance therapy, dose tapering, or discontinuation.

KNX100 was discovered through a comprehensive drug discovery program where it was initially selected for further development based on its ability to positively modulate social behavior in rodents and to activate key brain regions of interest, primarily the hypothalamus, as measured by c-fos immunohistochemistry. Kinoxis subsequently examined in vivo efficacy of KNX100 across a wide range of preclinical disease models including rodent and primate models of stimulant use disorders and rodent models of nicotine use disorder and OUD. In a C57BL/6 mouse model of opioid withdrawal, KNX100 was shown to dose dependently inhibit withdrawal-induced jumping (believe to be an escape behavior that captures the intense dysphoria being experienced during withdrawal) and paw tremors (a somatic symptom of opioid withdrawal).

Kinoxis has further evaluated the neural mechanisms causally involved in KNX100 effects on opioid withdrawal in mice. Using c-fos immunofluorescence to measure neural activation, it was observed that KNX100 inhibited opioid withdrawal-induced hyperactivation in 8 brain regions implicated in the opioid withdrawal syndrome: the lateral parabrachial nucleus, dorsal raphe, lateral periaqueductal gray, ventral tegmental area, medial division of the central amygdala, lateral habenula, nucleus accumbens shell (NAcSh), and ventral division of lateral septum. The NAcSh has been heavily implicated in opioid withdrawal. Subsequent studies using in vivo fiber photometry, which allows measurement of neural activation with millisecond precision in freely moving mice undergoing opioid withdrawal, demonstrated that KNX100 powerfully inhibited both the elevated baseline activity in the NAcSh and the withdrawal-jumping-related activity in this region. Further, it was also demonstrated that chemogenetic inhibition of the NAcSh inhibited opioid withdrawal-induced jumping in mice, and infusion of KNX100 directly into the NAcSh, at concentrations known to reach this brain region following oral administration in our efficacy dose range, inhibits withdrawal jumping. These data strongly indicate that KNX100 binding in the NAcSh, and subsequent inhibition of withdrawal-induced activity in this brain

region, plays a key role in its in vivo efficacy in reducing the severity of the opioid withdrawal syndrome in mice.

In nonclinical efficacy studies, KNX100 demonstrated significant activity in the mitigation of symptoms of opioid withdrawal in mice following naloxone induced precipitated withdrawal. In nonclinical studies, KNX100 also showed a favorable pharmacokinetic (PK) profile. It was rapidly absorbed in both rats and dogs, and the oral bioavailability appeared to increase dose dependently. Data obtained in the toxicity studies provided an acceptable safety profile and no-observed-adverse-effect levels (NOAELs) were clearly established and consistent across species.

Based on the favorable nonclinical evaluations performed to date (refer to the KNX100 Investigator's Brochure), Kinoxis is conducting this first-in-human (FIH) study to determine the safety and tolerability of single and multiple ascending doses of KNX100 in healthy volunteers.

2. STUDY OBJECTIVES

2.1. Primary Objectives

The primary objectives of this study are to evaluate the safety and tolerability of KNX100 administered orally as a single and multiple ascending doses in healthy volunteers.

2.2. Secondary Objectives

The secondary objectives of this study are to determine:

1. The PK profile of ascending doses of KNX100 and its metabolites when administered as a single oral dose in healthy volunteers.
2. The PK profile of ascending doses of KNX100 and its metabolites when administered as multiple doses in healthy volunteers.
3. The PK profile of KNX100 and its metabolites when administered twice daily as multiple doses in healthy volunteers.

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design and Plan

This is a Phase 1, FIH, single site, single treatment, 2-part, double blind, placebo controlled, randomized, single ascending dose (SAD)/multiple ascending dose (MAD) study designed to evaluate the safety and tolerability of KNX100 as compared to placebo. This study will enroll healthy volunteers.

The planned enrollment is approximately 64 male and female subjects. The total number of subjects will depend on the number of dose levels assessed in dose-escalation. Completed subjects will have received either KNX100 (active study drug) or placebo. Subjects who discontinue will be replaced to achieve the targeted number of evaluable subjects in each cohort.

Healthy subjects who meet all eligibility criteria will be sequentially enrolled and assigned to a single cohort in Cohorts 1-4 in Part A of the study or in Cohorts 1-3 in Part B of the study. Each cohort selected will evaluate 8 subjects; 6 subjects will be randomly assigned to receive KNX100, and 2 subjects will be randomly assigned to receive placebo. Each cohort will be enrolled sequentially, and the subsequent dose increase will be based on the Cohort Review Committee (CRC) evaluation of data known to date.

Additional cohorts may be added as required, based on safety profile and CRC authorization.

Part A (SAD) will initially dose 2 sentinel subjects (one with KNX100 and one with placebo) in Cohort 1. The center will review safety and tolerability up to and including the 48-hour timepoint for the sentinel subjects before dosing the remaining subjects in the dose cohort. The CRC will be informed and authorize the decision to continue with the remainder of the cohort dosing. Each subject will receive 1 dose, administered orally, of study drug. All subjects in each cohort will have their data, up to and including the 30 -hour postdose timepoint, evaluated for safety and tolerability by the CRC before opening the next dose cohort based on assessment of all available data. The dosing schedule may be extended if additional observations are needed at a particular cohort. In the absence of unacceptable AEs, the CRC may decide to open the next cohort before they have reviewed all data from all 8 evaluable subjects who have completed up to and including the 30-hour timepoint postdose.

Subjects will be housed in the clinic from Day -3 prior to study drug administration and will remain in-house for 30 hours postdose (Day 2) for PK and safety assessments.

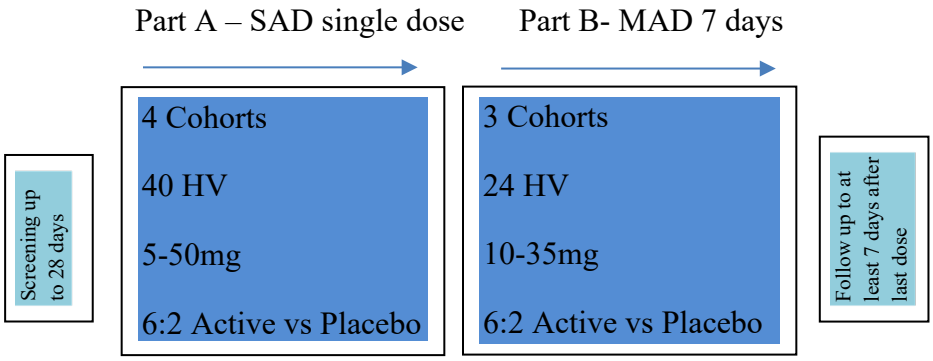
Part B (MAD) will evaluate up to 3 doses based on doses established during Part A (SAD). Each subject will receive 7 doses of study drug, administered either once (Cohorts 1 and 2) or twice daily (Cohort 3). All subjects in each MAD cohort will have their AE, PK, and electroencephalography (EEG) data, acquired up to and including Day 8, evaluated for safety and tolerability by the CRC before opening the next higher dose cohort. Subjects will be housed in the clinic from Day -3 prior to study drug administration and will remain in-house for 24 hours post morning dose (Day 8) for PK and safety assessments.

The study will be comprised of 3 periods: Screening (up to 28 days); Treatment (1 day for the SAD subjects and 7 days for MAD subjects) and Follow-up. Subjects will have a follow-up visit 7 days after the last dose. As SAD dosing is a single dose on Day 1, follow-up will occur

on Day 7. MAD dosing involves either single daily dosing (Cohorts 1 and 2) or twice daily dosing (Cohort 3) on Days 1 to 7, with a follow-up visit occurring on Day 14.

The overall study design is summarized in Figure 1. Refer to Appendix 1 and Appendix 2 for a detailed schedule of assessments.

Figure 1 Study Phases and Activities



3.2. Rationale for Study Design and Control Group

Study Design: This study is a 2-part, single site, single treatment, double-blind, randomized, placebo-controlled, dose escalating study to assess the safety, tolerability, and PK of single and multiple ascending doses and twice daily dosing of KNX100 administered orally to healthy males and females. Investigators and subjects will be blinded to treatment; however, the site pharmacist will be unblinded. Members of the CRC will be blinded; however, specific members may be unblinded to enable evaluation of PK and safety data for dose escalation decisions if required.

The design of this study is typical of those used for FIH studies with a "dose leader" (sentinel) design to better manage the safety aspects of the study. A sentinel design will be implemented during Part A, (SAD), with 2 subjects being dosed on the first dosing day of each Cohort. Of these, one will receive KNX100, and one will receive placebo. Safety and tolerability will be reviewed for these 2 sentinel subjects by the site, and CRC, before dosing the remaining subjects in the dose cohort.

All data for subjects in each SAD cohort collected up to and including the 30-hour samples postdose will be evaluated for safety and tolerability before opening the next higher dose cohort. In each MAD cohort, data for subjects up to and including the 24-hour timepoint post morning dose on Day 8 will be evaluated for safety and tolerability before opening the next higher dose cohort. The dosing schedule may be extended if additional observations are needed at a particular cohort. In the absence of unacceptable AEs, the CRC may decide to open the next

cohort before having reviewed data from all of the evaluable subjects who have completed Day 7 in the SAD cohorts and Day 14 in the MAD cohorts.

Dose: This study employs a SAD followed by a MAD design. Part A Cohort 1 will initially evaluate a low dose of KNX100 (5 mg). For the following cohorts (2-4), the KNX100 dose will be increased as agreed by the CRC up to a maximum of 50 mg of KNX100 (Table 3) or to the maximum exposure level defined in Section 6.20.1, whichever occurs first. In Part B (MAD), dosing will initially evaluate a low dose of 10 mg, followed by a high dose to be selected based on safety, tolerability and exposure data generated in Part A. Cohort 3 of Part B will evaluate twice daily (b.i.d) dosing. The dose will be selected based on PK data from the preceding cohort. KNX100 and placebo will be encapsulated in identical size 0 capsules to ensure blinding of study treatment. Additionally, subjects assigned to receive placebo will have the same number of capsules as subjects receiving KNX100. Predefined stopping rules are provided to ensure the safety of all study subjects are defined in Section 6.20.

Control: A matching placebo control will be administered to evaluate treatment-related effects.

Population: KNX100 has not been previously administered to humans; therefore, its effects in humans are as yet unknown. A healthy volunteer population with carefully considered inclusion/exclusion criteria will avoid the potential for interaction of KNX100 with any underlying disease state or concomitant medication that it may be necessary for subjects to take, while ensuring that subjects are fit and well enough for participation in the study. Females of child-bearing potential will have to undergo a pregnancy test prior to enrollment.

3.2.1. Starting Dose Justification

Animal doses (intraperitoneal [IP] and oral) assessed in primary pharmacology models were used to predict the human efficacious dose of oral KNX100. The efficacious doses administered to mice were converted to human dose equivalents per the *FDA Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers (July 2005)*.

Extensive preclinical efficacy data for the target indication of opioid withdrawal has been obtained using IP injection in mice. However, to date, preclinical efficacy data following oral administration in an opioid withdrawal mouse model is unavailable. The data collected in efficacy studies evaluating the effects of KNX100 (IP) on naloxone induced opioid withdrawal models in C57BL/6 mice and KNX100 (PO) in nicotine withdrawal models allow for a reasonable estimation of an effective dose range in humans, assuming allometric scaling on a mg/kg basis and similar affinities of KNX100 to pharmacodynamic (PD) targets in humans and C57BL/6 mice. Based on the available animal data, a pharmacologically effective dose range (PAD) of approximately 0.89-1.78 mg/kg of KNX100 phosphate (equivalent to 53-107 mg/day in a 60 kg adult) was anticipated for reducing the symptoms of opioid withdrawal in humans.

The human PK parameter estimates for CL/F and V/F based on single dose preclinical data from mouse, rat, and dog studies following oral administration was predicted using multiple allometric scaling approaches. Overall, the range of doses using multiple methods of prediction ranged from 53 to 125 mg. However, clearance in the rodent was much lower than in human, resulting in higher exposure levels that would not be achieved at the 53 or 125 mg doses. The AUC following a 3.7 mg/kg IP dose in the mouse was 23.3 h*ng/mL. Using the observed human CL/F value of 356,000 mL/h, a dose required to achieve the target AUC can be obtained using the following equation: Dose (ng) = CL (mL/h) × AUC (h×ng/mL). Using this formula, a total daily dose of 8.3 mg was derived. However, the C_{max} following the 3.7 mg/kg IP dose was 45.5 ng/mL. Based on the clinical data already obtained, a dose of around 20 mg could be estimated to achieve a C_{max} of 46 ng/mL.

The NOAEL reported in the 28-day repeat dose toxicology studies in rat and dog (Beagle) was 50 mg/kg/day in rat and 15 mg/kg/day in dog which is equivalent to a human equivalent dose (HED) of the free base equivalent of 8.1 mg/kg/day and 8.3 mg/kg/day, respectively. Based on a maximum recommended starting dose (MRSD) of $1/10^{th}$ of the NOAEL and using the most conservative approach, an MRSD of 0.81 mg/kg of free base which is equivalent to 1.2 mg KNX100 phosphate per kg per day. Assuming an average human weight of 60 kg, this would equate to a clinical starting dose of ~72 mg/day.

However, the severity, reversibility, and monitorable nature of the toxicity defining the NOAEL impacts how it influences the selection of the safe starting dose. Respiratory effects (decreased tidal and minute volume premonitory to decreased respiration rate in the rat) and central nervous system (CNS) effects (decreased body temperature and hypoactivity premonitory to convulsions in the rat; tremor, ataxia and hypoactivity premonitory to convulsions in the dog) both inform the selection of the safe starting dose. The severity of the CNS effects and trend towards greater-than-dose-proportional KNX100 exposure suggest a minimum of a 10-fold dose margin relative to the CNS effect threshold is appropriate to be protective of patient health. CNS effects, therefore, serve as the primary basis for selection of the starting and escalating clinical dose regimens.

The selection of the starting dose for human studies was based on the CNS NOEL determined in the 28-day dog toxicology study. In the dog, the CNS NOEL dose (7.5 mg free base/kg) corresponded to a HED of 6 mg KNX100 phosphate/kg. Therefore, $1/10^{th}$ of this value is equivalent to 0.6 mg KNX100 phosphate/kg, or 36 mg KNX100 phosphate in a 60 kg subject which would be the maximum recommended starting dose.

However, due to observation of convulsions and other CNS effects in the toxicology studies, Kinoxis commenced dosing at a dose lower than the calculated MRSD. Kinoxis defined the FIH starting dose as 5 mg KNX100 phosphate in a 60 kg subject. This starting dose is a more conservative dose than the toxicology program directs and predicted a C_{max} of 50 ng/mL and an AUC_{0-24} of 125 ng×hr/mL, which is approximately 30-fold lower than the exposure at the CNS NOEL in dogs, approximately 60- 90-fold lower than the exposure at which convulsions were observed in dogs. Based on the allometric body surface area (BSA) scaling approach, this starting dose was ~75-fold lower than the HED of the dog NOEL for convulsions reported in the 28-day toxicology study, 150-fold lower than the HED of the NOAEL in rats and dogs, and 250-fold lower than the dog LOEL for convulsions reported in the 28-day toxicology study.

Based on predictive modelling, the proposed starting dose of 5 mg was anticipated to achieve a maximum plasma concentration of approximately 50 ng/mL, well below the applied exposure cap. Subsequent clinical doses will be established based on scaling of the exposure results from the 5 mg dose.

3.2.2. Maximum Dose

An upper limit of 35 mg per day is supported by the nonclinical data and human PK data, specifically by exposure at the $1/10^{th}$ of the NOEL of 2.75 mg/kg established in the 14-day dog EEG study, however this will be guided by emerging safety and PK data. Dose escalation will stop at any time if unacceptable safety findings are identified or if the maximum concentration (C_{max}) or area under the curve (AUC) exceeds or is likely to exceed $1/10^{th}$ of the exposure associated with the NOEL of 2.75 mg/kg seen in the 14-day dog EEG study (84.9 ng/mL or 82.7

h×ng/mL for KNX100 and 26.5 ng/mL and 45.7 hr × ng/mL for KNX100A, respectively). In this case, the CRC may stop the study, or modify the dose downwards in subsequent cohorts.

3.3. Study Duration and Dates

This study is expected to run for approximately 12 months from the start of subject screening to the last subject finishing the study.

4. STUDY POPULATION SELECTION

4.1. Study Population

This study will enroll approximately 64 healthy volunteers at a single site in Australia.

This clinical study can fulfill its objectives only if appropriate subjects are enrolled. The following eligibility criteria are designed to select healthy subjects for whom protocol treatment and procedures are considered appropriate. All relevant medical and non-medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular subject.

4.2. Inclusion Criteria

Subjects will be eligible for participation in the study only if they meet ALL of the following inclusion criteria.

None of the inclusion criteria are eligible for re-screening. No waivers will be granted and any deviation from below will be recorded as a major protocol deviation. An exception can apply for subjects who meet the inclusion and exclusion criteria, but then miss a cohort because it is filled. This would mean they are no longer eligible due to the 28-day screening window lapse. These are the only subjects that may be rescreened.

1. Ability to understand and provide written informed consent.
2. Body mass index (BMI) within the range of 18-32 (inclusive).
3. Healthy male and female volunteers ≥18 and ≤55 years old at Screening.
4. Able and willing to comply with the requirements of the study and complete the full sequence of protocol related doses, procedures, and evaluations.
5. Willing to agree not to use alcohol or recreational drugs and willing to have drug screening, prior to the first dose of KNX100 and if drug use is suspected while active in the study.
6. Willing to agree not to smoke cigarettes or use tobacco-based products prior to the first dose of KNX100 and for the entire duration of the study.

7. Males who are sexually active must use a condom OR be abstinent OR have the same sex partner OR be surgically sterile OR have partner who is of non-childbearing potential, for at least 90 days after the last dose of investigational drug. If female partner is a Woman of Child-Bearing Potential (WOCBP), the female partner must use highly effective methods of contraception, defined as below:
    - Hormonal methods of contraception including oral contraceptives containing combined estrogen and progesterone, a vaginal ring, injectable and implantable hormonal contraceptives, intrauterine hormone-releasing system (e.g., Mirena) and progestogen-only hormonal contraception associated with inhibition of ovulation.
    - Nonhormonal intrauterine device,
    - Bilateral tubal occlusion.

Refer to Section 6.9 for further detail on contraception.

4.3. Exclusion Criteria

Subjects will **not** be eligible for participation in the study if they meet ANY of the following exclusion criteria.

1. Clinically significant history or presence of significant cardiovascular, respiratory, hepatic, renal, gastrointestinal, endocrinological, hematological, neurological, or psychiatric disorder. Any surgical or medical history which may significantly alter the absorption, metabolism, or elimination of drugs or constitute a risk when taking the study intervention; or interfering with the interpretation of data (e.g., gastric bypass, cyclical vomiting, etc.). This includes a history of lymphoma, leukemia, or any malignancy within 5 years except for basal cell or squamous epithelial carcinomas of the skin that have been resected with no evidence of metastatic disease for 3 years.
2. Subjects who have a sitting or semi-supine blood pressure at screening or Day-1, after resting for at least 3 minutes of systolic blood pressure >140 or <100 mmHg, or diastolic blood pressure >90 or <60 mmHg.
3. Subjects who have a sitting or semi-supine pulse rate at screening or Day-1, after resting for at least 3 minutes, outside the range of <50 or >90 beats/minute
4. Subjects who donated blood or who had a comparable blood loss (approximately 500 mL) during the last 30 days prior to start of this study and while on study.
5. Clinically significant findings on the screening, Day -1, or predose Day 1 electrocardiogram (ECG) or physical examination, including QTcF duration >450 ms for males and >470 ms for females on ECG.
6. Thyroid function tests outside the normal reference ranges and deemed clinically significant by the study PI (and upon repeat).

7. Safety laboratory tests that are outside the normal reference ranges and deemed clinically significant by the study PI (and upon repeat).
8. Any history of meningitis, septicemia, or pneumonia.
9. Any history or family history (first or second degree relative) of seizure disorder, febrile convulsions.
10. Any clinically significant medical history of closed head trauma.
11. Any history of anaphylaxis or other significant allergy.
12. Any current diagnosis or clinically significant medical history of psychiatric illness as diagnosed and documented by a medical practitioner and as defined by the American Psychiatric Association Diagnostic and statistical manual of mental disorders 5th edition (DSM-5).
13. Subjects with a history of chronic alcohol (regular daily intake of more than three standard drinks) or drug abuse within the last 6 months prior to first administration, or evidence of such abuse as indicated by the laboratory profile conducted during the screening examination.
14. Subjects who have received prescription drugs or over-the-counter (OTC) medication including dietary supplements, COVID-19 vaccine, standard dose vitamins, or herbal products within 14 days prior to the first administration (with the exception of the oral contraceptive pill).
15. Subjects who received any treatment agents known to alter the major organs or systems within 30 days prior to the first administration (e.g., diuretics, nephro- or liver toxic medication, barbiturates, phenothiazines, cimetidine, more than 1.0 L of caffeine-containing beverages per day, etc.).
16. Diagnosed infection of any kind, e.g., viral, bacterial, fungal, or mycobacterial within 1 month prior to the first dose of KNX100 or current fever or clinical signs or symptoms of infection at screening or Day -1.
17. Treatment with an unapproved investigational therapeutic agent within 30 days (or 5-half-lives for small molecule agents) prior to the first dose of KNX100.
18. Females who are pregnant (positive pregnancy test at screening or prior to first dose), lactating or unable/unwilling to use defined methods of contraception throughout the study.

Laboratory assessments conducted at screening for any exclusion criterion may be repeated once to rule out a laboratory error. Only the value leading to inclusion of the subject will be recorded. Vital signs recorded outside the exclusion criteria ranges may also be repeated once for confirmation.

5. STUDY TREATMENTS

5.1. Description of Treatments

5.1.1. Study Drug

KNX100 will be provided in capsule form as either 5 mg, 25 mg, or 100 mg capsules (Table 2).

Table 2 Dose Strength Per Capsule

Study Drug	Dosage Strength/Dosage Form		Manufacturer
	KNX100 Phosphate	Free Base Equivalent	
KNX100	5 mg/capsule	3.4 mg/capsule	Quotient Sciences (formerly Arcinova Ltd).
KNX100	25 mg/capsule	16.8 mg/capsule	Quotient Sciences (formerly Arcinova Ltd)
KNX100	100 mg/capsule	67.1 mg/capsule	Quotient Sciences (formerly Arcinova Ltd)

5.1.2. Placebo

KNX100 matching placebo will be provided in capsule form. An equivalent number of capsules will be administered to subjects receiving placebo (e.g., the first dose of study drug will be one 5 mg capsule; subjects receiving placebo will also receive only one capsule).

5.2. Treatments Administered

Both KNX100 and placebo will be administered orally based on the assigned treatment group (refer to Section 5.3). For each cohort, a total of 8 subjects will be dosed (6 with KNX100 and 2 with placebo).

Part A will evaluate doses of KNX100 starting with 5 mg and increasing up to a maximum of 50 mg per day (Table 3). Doses post the 5 mg dose will be decided by the CRC upon review of all available data.

Part B will evaluate a low- (10 mg), and high-dose, based on the doses evaluated in Part A and the safety, tolerability, and PK of doses in the previous cohorts, administered for 7 consecutive days (Table 4). Part B (Cohort 3) will evaluate twice daily dosing of a single dose, selected based on the safety, tolerability, and PK of doses in the previous cohorts, administered for 7 consecutive days (Table 4).

5.3. Selection and Timing of Dose for Each Subject

Pertinent information for the SAD study is provided in Section 5.3.2, along with the proposed dose escalation schedule in Table 3.

Pertinent information for Part B is provided in Section 5.3.3, along with the proposed dose escalation schedule in Table 4.

Subjects in both Part A (SAD) and Part B (MAD) phases will be housed in the clinic from Day - 3 prior to each dose and remain in-house for 30 hours postdose (Day 2) for Part A and 8 days post initial dose for Part B (Day 8).

5.3.1. Sentinel Subject Review and Toxicity Management

Part A Cohorts 1 through 4 will initiate dosing with 2 sentinel subjects who are randomly assigned so that 1 subject receives KNX100 and the other receives placebo. Following dosing of study medication, site personnel will observe the sentinel subjects for 48 hours, at which time the investigator will review the safety and tolerability for both sentinel subjects. This will include housing of the sentinel subjects in the Phase 1 unit for 30 hours postdose and then a follow up phone call at approximately 48 hours postdose administration.

Data reviewed will include AEs, clinical laboratory results, vital signs, and ECGs, EEGs, and PK parameters for both subjects. PK will be evaluated to ensure that exposure has not exceeded the maximum clinical exposure cap defined in Section 6.20.1. Upon acceptance of reviewed data by the site and CRC, dosing may continue for the remaining 6 subjects. The investigator will document the review and decision by email to Sponsor representatives.

5.3.2. Part A Dosing

Study drug will be administered once on Day 1. Part A dosing will commence with 2 sentinel subjects prior to dosing the remainder of each cohort (refer to Section 5.3.1).

Study drug will be administered in the morning together with a glass of tap water under a fasted state, i.e., after an overnight fast of at least 10 hours and no food is allowed for at least 2 hours after drug administration. Please refer to Section 5.10.3 for information related to fluid and food restrictions both pre- and postdose.

Table 3 provides the planned dose-escalation schedule. Of note, dose escalations will not exceed dose-doubling in principle.

Table 3 Planned Dose-Escalation Schedule (SAD Part A)

Cohort	Oral Daily Dose (mg/day)[1]		Total Number of Capsules (Number - Strength)[1]	
	KNX100	Placebo	KNX100	Placebo[2]
1	5	0	1 capsule (1 × 5 mg)	1 capsule (1 × 0 mg)
2	15	0	3 capsules (3 × 5 mg)	3 capsules (3 × 0 mg)
3	25	0	25 mg: 1 capsule (1 × 25 mg)	25 mg: 1 capsule (1 × 0 mg)
4	50	0	50 mg: 2 capsules (2 × 25 mg)	50 mg: 2 capsules (2 × 0 mg)

Abbreviations: PK = pharmacokinetics.

[1] All dose levels beyond Cohort 1 may change based on emerging safety and PK data.

[2] An equivalent number of capsules will be administered to subjects receiving placebo.

*Additional cohorts may be added as required, based on safety profile and CRC authorization.

Dose escalation and de-escalation will follow the processes outlined in Section 5.3.4.

5.3.3. Part B Dosing

In Cohorts 1 and 2, study drug will be administered once daily for 7 days at approximately the same time each day ± 30 minutes. In Cohort 3, study drug will be administered twice daily, approximately 8 hours (± 30 minutes) apart for 7 days. Doses will be administered:

- Morning (e.g., at 11:00 am) at approximately the same time (± 30 minutes) each day
- Afternoon/ Evening (e.g., at 7:00 pm) at approximately the same time (± 30 minutes) each day

The first dose of study drug will be administered together with a glass of tap water under a fasted state i.e., after an overnight fast of at least 10 hours. For MAD Cohort 3, the second dose of study drug will be administered together with a glass of tap water under a fasted state i.e., after fasting for at least 2 hours prior to dose administration. For all cohorts, no food is allowed for at least 2 hours after drug administration. Please refer to Section 5.10.3 for information related to fluid and food restrictions both pre- and postdose.

Table 4 provides the planned dose-escalation schedule for Part B (MAD). Up to 2 dose strengths ranging from as low as 10 mg and up to the clinical exposure cap will be selected based on the tolerability and pharmacokinetics of the previous dose.

Table 4 Planned Dose-Escalation Schedule (MAD Part B)

Cohort	Oral Daily Dose (mg/day)[1]		Total Number of Capsules/Day (Number - Strength)[1]	
	KNX100	Placebo	KNX100	Placebo[2]
1	10 (Low)	0	2 capsules (2 × 5 mg)	2 capsules (2 × 0 mg)
2	30 (High)	0	2 capsules (1 × 5 mg, 1 × 25 mg)	2 capsules (2 × 0 mg)
3	30 (bid) [3]	0	2 capsules (1 × 5 mg, 1 × 25 mg) (am) 2 capsules (1 × 5 mg, 1 × 25 mg) (pm)	2 capsules (2 × 0 mg) (am) 2 capsules (2 × 0 mg) (pm)

Abbreviations: am = morning; bid = twice daily; MAD = multiple ascending dose; pm = afternoon/evening

[1] Dose levels will be based on data from Part A and emerging PK data.

[2] An equivalent number of capsules will be administered to subjects receiving placebo.

[3] Dose to be administered twice daily, 8 hours apart (refer also to Section 5.3.3).

5.3.4. Dose Escalation

An evaluable subject is one that has been dosed, completed all assessments, and had their data reviewed by the CRC.

Blinded data to be reviewed by the CRC for each subject will include (as a minimum): PK (up to 30 hours postdose for Part A and 24 hours postdose for Part B), EEG, ECG, vital signs, pulse oximetry, neurological examination, and any adverse events.

The CRC may approve dose escalation following review of cohort data if:

- No drug related SAE's occurring within 48 hours of administration of KNX100 or placebo is observed in any subject in the dose group under evaluation.
- No drug related AE's of Grade 3 or greater are observed in 2 or more subjects within a dose group.
- The mean KNX100 C_{max} **OR** AUC does not exceed 84.9 ng/mL or 82.7 ng×h/mL respectively **and** the mean KNX100A C_{max} **OR** AUC does not exceed 26.5 ng/mL and 45.7 hr × ng/mL, respectively, the exposure associated with $1/10^{th}$ of the NOEL of 2.75 mg/kg observed in the 14-day dog EEG study.
- No emergence or evidence of other PI-deemed and or medical monitor-deemed clinically significant abnormal vital signs, neurological and physical examination, or safety parameters postdose have been observed.

Dose increases will be permitted once review of data from all evaluable subjects has been performed.

5.4. Method of Assigning Subjects to Treatment Groups

Subjects will be assigned to a cohort based on the order in which they are enrolled. Subjects will be centrally randomized to treatment in a 6:2 ratio within each assigned cohort. The first 2 sentinel subjects will be assigned to treatment in a 1:1 ratio such that 1 subject receives KNX100 and the other receives placebo. Once eligibility is re-confirmed (Day -1), subjects will be randomized via a randomization list or an Interactive Web Response System. Of note, after Cohort 1, the CRC will determine the dose to be administered; therefore, randomization codes will only indicate whether the subject will receive active or placebo within their cohort.

Subjects will be sequentially assigned a Subject identification number upon signing the informed consent (i.e., site number 10, Screening number 001, 002 = Subject number 10-001, 10-002 etc.). Subjects will be identified by their Subject identification number throughout the study. Subjects will receive a 4-digit randomization number on Day 1 before dosing Replacement Subjects.

Subjects can be replaced. Replacement subjects will receive the same randomization number of the replaced subject +1000 (e.g., Subject 1111 will replace Subject 0111, Subject 1211 will replace Subject 0211, etc.). If more than one replacement becomes necessary, additional replacements will be numbered 2XXX, 3XXX, and so on (Table 5). The replacement subject will receive the treatment assigned to the withdrawn subject.

Table 5 Example 4-Digit Subject Randomization Numbers

Cohort	Original Subject Randomization Numbers	Replacement Subject Randomization Numbers
Part A		
Cohort 1	0111-0118	1111-1118, 2111-2118, 3111-3118, etc.
Cohort 2	0121-0128	1121-1128, 2121-2128, 3121-3128, etc.
Cohort 3	0131-0138	1131-1138, 2131-2138, 3131-3138, etc.
Cohort 4	0141-0148	1141-1148, 2141-2148, 3141-3148, etc.
Part B		
Cohort 1M	0211-0218	1211-1218, 2211-2218, 3211-3218, etc.
Cohort 2M	0221-0228	1221-1228, 2221-2228, 3221-3228, etc.
Cohort 3M	0231-0238	1231-1238, 2231-2238, 3231-3238, etc.

5.5. Blinding

The study will be performed in a double-blind fashion. The investigator and study staff (including lab personnel), the subjects, the monitors and the Sponsor's staff will remain blinded to the treatment until database lock.

The investigator will receive sealed envelopes with the individual randomization per subject to be opened in the case of emergency only.

The pharmacist of the site will receive the randomization list to enable packaging.

The randomization code will be kept strictly confidential by the pharmacist of the site. Double-blind conditions will be established so that the investigator, site personnel and subjects are blinded to the treatment assignments. Parameters to achieve and maintain the double-blind status of the study include:

- Sequential assignment of subject numbers within each cohort.
- Labeling of study drug with the study number.
- Packaging and delivery of study drug supplies to sites in a manner that maintains blinding.
- Matched appearance of study drug and placebo,

Subjects, investigators, and clinical staff will be blinded to therapy assignment. Sponsor representatives involved in the conduct of the study will be blinded to therapy assignment. The site pharmacy staff will be unblinded to study treatment.

To ensure study blinding, the active and placebo will be provided in identical capsules and an equivalent number of capsules will be administered to subjects receiving placebo (refer to Section 5.3.2 and Section 5.3.3).

The following roles may be unblinded:

- Unblinded biostatistics team that prepares the randomization materials and handles treatment-revealing data prior to database lock.
- Selected study Sponsor personnel who are not directly involved in the conduct of the study.
- Pharmacy team.
- Drug-reconciliation clinical research associate (CRA).

A list of unblinded individuals will be maintained in study files. The pharmacy team will prepare the investigational product dispensing but will have no other role in the study and will not discuss any study-related observations with the blinded team members.

Analysis of the safety data for dose escalation decisions will be performed on blinded data and in a masked fashion. Analysis of PK parameters will be evaluated by members of the CRC. Please refer to Section 9.8 for additional information.

5.6. Unblinding

The investigator and site personnel will remain blinded to the randomization code throughout the duration of the study. When necessary, code break envelopes will be available 24 hours a day, 7 days a week to unblind a treatment assignment.

Treatment assignment for an individual subject should be unblinded by the investigator only in an emergency, and only if knowledge of the treatment assignment is necessary for the clinical management or welfare of the subject. If possible, the investigator should contact the medical monitor before unblinding, but priority should be given to safety of the subject. The investigator must record the date and reason for unblinding.

Subjects may be unblinded in the event that an AE of Grade 3 or greater is reported in 2 or more subjects to support CRC data review and decisions regarding dose escalation or stopping criteria.

If deemed to be necessary, the CRC may be required to break the randomization code. Since the investigator is a member of the CRC, this will be performed by the investigator.

5.7. Missed Doses

During Part A (SAD), study subjects will receive only 1 dose of product; therefore, missed doses will not be an issue.

During Part B (MAD), missed doses will be recorded in the electronic case report form (eCRF) along with the reason. During the MAD phase, missing 2 or more doses will constitute a protocol violation. Such study subjects may be replaced with due consideration of circumstances by the study CRC (refer to Section 9.8).

5.8. Overdose

An accidental overdose is highly unlikely because a licensed, unblinded pharmacist will dispense KNX100. In the event of an accidental overdose, the site shall contact the medical monitor immediately to discuss an appropriate course of action and follow-up.

5.9. Concomitant Therapy

All therapy ongoing at the time of enrollment and all therapy (other than study drug) received during the study is considered concomitant therapy for purposes of this study.

Intake of prescription or nonprescription is prohibited within 14 days or 5 half-lives of the drug, whichever is longer, prior to the first dose study medication, until completion of the follow-up visit.

Investigators may prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care. All concomitant medications taken during the study will be recorded with indication, dose information, and dates of administration.

Subjects cannot be enrolled if they have received prescription drugs or OTC medication including dietary supplements, standard dose vitamins, or herbal products within 14 days prior to the first administration (with the exception the oral contraceptive pill).

5.10. Restrictions

As this study is being conducted in healthy volunteers and, to date, no drug-drug interactions have been identified, no prohibited concomitant therapy has been defined.

5.10.1. Smoking

Subjects must be non-smokers or willing to abstain from smoking or using tobacco products from Day -1 throughout the entirety of the study.

5.10.2. Prior Therapy

Restrictions for prior therapy are as noted in Section 4.3.

5.10.3. Fluid and Food Intake

While in the study clinic, subjects will not be allowed to consume any food and beverages not provided by the institution. Standardized meals will be provided for all study subjects.

During each dosing session, subjects will abstain from ingesting caffeine or xanthine-containing products (e.g., coffee, tea, cola drinks, and chocolate) for 10 hours before the start of morning dosing until after collection of the final PK and/or PD sample.

Use of alcohol will not be allowed from screening until after the EOS/ET visit.

Subjects should fast overnight prior to screening and for 2 hours prior to administration of a second dose of study drug (MAD Cohort 3). Subjects should avoid consumption of food for up to 2 hours following administration of all doses of study drug.

5.10.4. Exercise

Subjects must abstain from strenuous physical activity for 48 hours prior to admission until discharge on Day 2 (SAD) or Day 8 (MAD).

5.11. Treatment Compliance

Site personnel will administer study drug; therefore, treatment compliance is not a concern for this study.

5.12. Packaging and Labeling

All study treatments will be manufactured, packaged, and labeled under GMP conditions, and will be supplied to the clinical site pharmacy as unblinded study products. A certificate of analysis will be provided for each strength.

Study drug (KNX100 and placebo) will be encapsulated in hydroxypropyl methylcellulose (HPMC) dark green opaque size 0 capsules and packaged in 100 mL high density polyethylene (HDPE) Duma Twist-off bottles with polypropylene twist-off, 45 mm closures.

Each bottle will have a label that will contain, at a minimum, the protocol number, lot number, name and strength of the product, quantity of dosage units, directions for use, storage conditions, country-specific regulatory caution statement, and name and address of the Sponsor.

5.13. Storage and Accountability

All study drug must be kept in an appropriate, limited-access, secure place until it is used or returned to the Sponsor or designee for destruction. All Sponsor-supplied drugs must be stored under the conditions specified on the label and remain in the original container until dispensed.

Study drug should be stored at room temperature conditions (25°C [77°F]); with excursions permitted to 15°C-30°C [59°F-86°F], refer to USP Packaging and Storage Requirements).

Upon receipt of the study drug, the investigator or designee must verify the contents of the shipments against the packing list. The verifier should ensure that the quantity is correct, that the medication is received within the labeled storage conditions, and that the package is in good condition. If quantity and conditions are acceptable, the investigator or designee should acknowledge the receipt of the shipment. If there are any discrepancies between the packing list versus the actual product received, Kinoxis must be contacted to resolve the issue. The packing list should be filed in the investigator's essential document file.

The site must maintain 100% accountability for all study drugs received and dispensed during the entire participation in the study. Proper drug accountability includes, but is not limited to:

- Frequently verifying that actual inventory matches documented inventory.
- Verifying that the log is completed for the drug lot (or ID or lot number) used to prepare each dose.

- Verifying that all containers used and unused are documented accurately on the log.
- Verifying that required fields are accurately completed and are legible.

The investigator should ensure that study drug is used only in accordance with the approved protocol. Study drug shall be dispensed only to subjects enrolled in the study. If any dispensing errors or discrepancies are discovered, the Sponsor must be notified immediately.

The site must record the current study inventory on a Sponsor-approved drug accountability log. At a minimum, the following information will be recorded for each subject:

- Protocol number and title.
- Name of investigator.
- Site identifier and number.
- Description of Sponsor-supplied drugs.
- Date and amount dispensed, including initials of the person dispensing the drug.
- Date and amount returned as unused including the initials of the person receiving the Sponsor-supplied drug.

The log should include a separate entry for each subject who receives Sponsor-supplied study drug.

Prior to site closure or at appropriate intervals, a representative from the Sponsor or its designee will perform study material accountability and reconciliation before study materials are returned to the Sponsor or its designee for destruction. The investigator will retain the original documentation regarding study material accountability and return of study materials; copies (as required) will be sent to the Sponsor or designee.

The investigator will be notified of any expiry date or retest date extension of study material during the study conduct. On expiry date notification from the Sponsor or designee, the site must complete all instructions outlined in the notification, including segregation of expired study material for return to the Sponsor or its designee for destruction.

6. STUDY PROCEDURES

6.1. Information Statement and Consent Form

Subjects who are identified as potentially suitable to participate in the study will be sent an information statement and consent form as approved by the Sponsor and by the Human Research Ethics Committee (HREC). The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation.

The screening visit will provide an additional opportunity for the subject to discuss any questions that they may have with study staff. Study staff are responsible for ensuring that the subject receives adequate information, and all questions are answered appropriately. Additionally, study staff must ensure subjects comprehend the information provided about the study, including a decision to participate will not impact usual medical care, and subjects retain an absolute right to withdraw at any time for any reason. If, after this discussion, the subject still wishes to enroll, the staff member that conducts the consent discussion will ensure that the consent is documented appropriately.

Documentation of consent must be obtained before any study-specific procedures are performed. A copy of the signed consent form will be given to the subject. The date that consent was obtained will be recorded in the electronic case report form (eCRF) as well as the subject's chart. Following documentation of consent, the subject will be allocated a screening number and will undergo screening assessments as indicated in Appendix 1 and Appendix 2 to ensure eligibility.

6.2. Randomization

On Day -1, the inclusion/exclusion criteria will be reviewed with the subject. A urine pregnancy test will be performed for WOCBP and subjects will be screened for drugs of abuse via a test panel upon admission (Day -3). Prior and concomitant medications will be reviewed. Any AEs since the signing of the informed consent will be collected. Please also refer to Appendix 1 and Appendix 2.

If the subject still meets study criteria, they will be randomized prior to dosing.

6.3. Demographic and Baseline Characteristics

Demographic data and other baseline characteristics will be recorded, including date of birth or age, gender, self-reported race and/or ethnicity, and smoking status of the subject. A standard medical, medication, and surgical history will be obtained with review of the selection criteria with the subject.

A medical history will be collected during screening using forms designed to capture, at a minimum, the parameters specified by the protocol and to be recorded in the study case report form (CRF). Medical history includes an assessment of past and present clinically significant diseases and surgeries or important medical events (any within the past year). Additionally, an assessment of current reproductive status will be collected.

Record all medications (e.g., prescription and OTC medications, hormonal birth control, herbal or homeopathic remedies, and nutritional supplements) used by the subject within 30 days prior to the screening visit.

Ongoing medical status will also be reviewed at each study visit to screen for and capture AEs.

6.4. Physical and Neurological Examinations

A medical assessment will be completed by the principal investigator (PI) or a study physician at study entry, and at any early withdrawal visits. The primary purpose of the medical assessment at study entry is to confirm the subject's suitability to enroll in the study.

The physical examination will include height (screening only), body weight, and an evaluation of the head, eyes, ears, nose, and throat, and the cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, and neurological systems. Record any abnormality identified prior to administration of study medication as medical history.

Any clinically significant, new, or worsened abnormalities that occur after administration of study medication should be recorded as AEs. Additional parameters may be assessed as indicated by any subject symptoms.

Abbreviated physical examinations performed throughout the study should be symptom-directed.

Neurological examination shall include assessment of cranial nerves, motor, sensation, coordination, reflexes, and gait. Abbreviated neurological examinations performed throughout the study include light touch/power in limbs and brief cranial nerve examination.

Findings will be recorded as normal/abnormal for each parameter with additional description as indicated. All abnormal, clinically significant, findings will be considered AEs.

Please refer to the Schedule of Assessments (SOA) in Appendix 1 and Appendix 2 for details regarding timing for assessments.

6.5. Vital Signs

Vital signs will be recorded at screening, study entry, and at all visits before and after dosing of study drug, including:

- body temperature,
- respiratory parameters (including respiratory rate, saturated oxygen, pulse oximetry),
- pulse rate,
- heart rate (after 3-minute semi-supine rest), and
- arterial blood pressure (systolic and diastolic; after 3-minute rest in either a semi-supine or sitting position).
- peak flow measurement.

During the inpatient dosing period, vital signs also include respiratory rate, continuous pulse oximetry and saturated oxygen, which will be undertaken via telemetry within 2 hours predose and at 2 hours postdose (± 15 minutes) as per the schedule in Appendix 1 and Appendix 2. For MAD Cohort 3, vital signs, including telemetry will be conducted following each dose (am and pm).

Peak flow measurement will also be undertaken within 1 hour predose as well as at 1-, 2-, and 4-hours (± 15 minutes) postdose. For MAD Cohort 3, peak flow measurements will be conducted following each dose (am and pm). The highest of 3 peak flow measurements, at each timepoint shall be recorded.

Protocol Exclusions 2 and 3 pertaining to vital signs should be checked at both Screening and Day -1 to confirm eligibility. Following the Day-1 eligibility check, any subject with vital signs outside of acceptable range will have their vital signs repeated. If the readings are again abnormal, but not clinically significant, it is at the PI's discretion as to whether to continue the subject in the study. Normal vital sign ranges for this study are presented in Appendix 4.

For details regarding timing for assessments, please refer to the SOA in Appendix 1 and Appendix 2.

6.6. Gastrointestinal Symptoms

Changes in gastrointestinal function will be monitored during the MAD part of the study. Bowel movements, such as constipation, and any change in bowel habits as reported by study subjects will be recorded daily during the dosing period.

Please refer to the SOA in Appendix 2 for details regarding timing for these assessments.

6.7. Columbia Suicide Severity Rating Scale (C-SSRS)

The C-SSRS is a questionnaire used for suicidality assessment developed by multiple institutions, including Columbia University. The scale is evidence-supported and is part of a US national and international public health initiative involving the assessment of suicidality. The scale has been successfully implemented across many settings, including schools, college campuses, military, fire departments, the justice system, primary care and for scientific research. The C-SSRS Risk Assessment version is intended to help establish a person's immediate risk of suicide and is used in acute care settings.

The C-SSRS will be administered at screening and again at Day 4 and Day 7 postdose during the MAD part of the study. Please refer to the SOA in Appendix 2 for details regarding timing for assessments.

6.8. Concomitant Medications

Concomitant medications will be assessed at each study visit by staff discussion with the study subjects. For any concomitant medications not otherwise linked to reported AEs, staff should discuss with the subject to clarify the indication for medication.

Refer to Section 5.9 for information on prior and concomitant medications, including those that must be halted within defined time periods prior to randomization.

Please refer to the SOA in Appendix 1 and Appendix 2 for details regarding timing for assessments.

COVID-19 vaccinations will be permitted if they occur more than 14 days before the treatment period or after the EOS visit.

6.9. Contraception

WOCBP must have a negative serum or urine pregnancy test within 24 hours prior to the start of study drug and must not be breastfeeding, lactating, or planning pregnancy during the study period. WOCBP are defined as any female who has experienced menarche and who has not undergone surgical sterilization (hysterectomy or bilateral oophorectomy) and is not postmenopausal. Menopause is defined as 12 months of amenorrhea in the absence of other biological causes. In addition, females under the age of 55 years must have a documented serum follicle stimulating hormone (FSH) level > 40 mIU/mL to confirm menopause. Male participants with potentially postmenopausal partners who are under the age of 55 years must use condoms unless their partner's postmenopausal status has been confirmed by FSH level.

WOCBP who are not exclusively in same-sex relationships and males with partners of child-bearing potential must agree to use adequate contraception. For WOCBP, contraception should be continued for 30 days after the final study visit. For males, contraception should be continued for 90 days after the final dose of study drug. In addition, males must not donate sperm for 90 days after the final dose.

Males and WOCBP who are continuously not heterosexually active are exempt from contraceptive requirements. However, WOCBP must still undergo pregnancy testing as per protocol.

Investigators will counsel WOCBP and male participants who are sexually active with WOCBP on the importance of pregnancy prevention and the implications of an unexpected pregnancy. Investigators will advise on the use of an adequate methods of contraception, which is defined as use of a condom by the male partner combined with use of a highly effective method of

contraception by the female partner. A highly effective method of contraception is one that has a failure rate of <1% when used consistently and correctly. Male participants must inform their female partners who are WOCBP of the contraceptive requirements of the protocol and are expected to adhere to using contraception with their partner.

Highly effective methods of contraception are listed below:

- Hormonal methods of contraception including oral contraceptives containing combined estrogen and progesterone, a vaginal ring, injectable and implantable hormonal contraceptives, intrauterine hormone-releasing system (e.g., Mirena) and progestogen-only hormonal contraception associated with inhibition of ovulation.
- Nonhormonal intrauterine device (IUD)
- Bilateral tubal occlusion
- Vasectomized subject/partner with documented azoospermia 90 days after procedure if that partner is the sole sexual partner.

For female participants, hormonal contraceptives should begin at least 1 month prior to screening to ensure contraceptive is in full effect.

Complete abstinence, defined as the complete avoidance of heterosexual intercourse, is an acceptable form of contraception if used consistently throughout the duration of study and for the durations after dosing specified for males and females above. It is not necessary to use any other method of contraception when complete abstinence is elected. WOCBP who choose complete abstinence must continue to have pregnancy tests as per protocol. The reliability of sexual abstinence needs to be evaluated by the investigator in relation to the duration of the clinical study and the preferred and usual lifestyle of the subject.

6.10. 12-Lead Electrocardiograms

12-lead ECGs will be used to evaluate heart rate, atrial ventricular conduction, QRS and QT intervals, and possible arrhythmias. ECGs include ventricular heart rate; QRS duration; PR, RR, QT, QTcB, and QTcF intervals; and P and T wave. Evaluations will be recorded as normal, abnormal (not clinically significant), or abnormal (clinically significant).

The 12-lead ECGs will be obtained after the subject has been resting semi-supine for at least 3 minutes prior to the indicated times. All ECGs should be recorded with the subject in the same physical position. For each time point, 3 ECG recordings should be taken at approximately 1 to 3-minute intervals.

A standardized ECG machine should be used, and the subject should be tested using the same machine throughout the study, if possible. When the timing of these measurements coincides

with a blood collection, obtain the ECG prior to the nominal time of the blood collection, blood pressure, and heart rate.

Additional ECGs should also be collected if a subject experiences a cardiac AE.

After ECGs have been recorded, the PI or a designated physician will review each of the ECGs. If available, a paper copy should be filed in the subject's medical records. For all ECGs, ECG intervals and an overall assessment will be recorded. Evaluations will be recorded as normal, abnormal (not clinically significant), or abnormal (clinically significant).

For details regarding timing for assessments, please refer to the SOA in Appendix 1 and Appendix 2.

6.11. Electroencephalograms

6.11.1. Screening and Baseline Procedures and Assessments

Sleep deprived EEG's shall be conducted during screening and shall be utilized for excluding patients at increased seizure risk, predicting overall seizure risk, informing dose escalation decisions once dosing has commenced, and contributing to the overall safety monitoring program. These EEGs shall also be used as a baseline to facilitate detection of changes in future EEGs completed throughout the study. Screening EEG's should be conducted only after all other Screening activities have completed. Subjects previously consented or screened for KTX-101 who have undertaken a previous sleep-deprived EEG within the last 12 months, will not have to have this procedure repeated.

Subjects will be admitted to the clinic at Day -3 to undertake a sleep deprived EEG on Day -2. Subjects will be requested to maintain their usual sleep routine, however, only sleep for 4 hours (± 1 hour) prior to undertaking the 1-hour screening EEG. The EEG recording will be reviewed to identify any IEDs or electrographic seizures. If present, the subject will be excluded and be discharged from the unit. Volunteers that display this increased risk are referred to an epilepsy clinic for specialist follow-up.

During the study, 24-hour continuous EEG recordings will also be reviewed as a safety assessment for any IED's pre and postdose (as a marker of increased susceptibility postdose).

EEG data will form part of the CRC data review package from each cohort (including sentinels and rest of cohort dosing) to mitigate seizure potential and allow safe dose escalation. The presence of epileptiform discharges observed on the EEG recordings will also contribute to the stopping rules criteria and, if present, no further dose escalation will occur.

6.11.2. Dosing Phase Assessments

During the SAD phase, subjects will undertake a continuous ambulatory EEG from at least 30 minutes predose to 24 hours (±1 hour) after administration of their dose.

Please refer to the SOA in Appendix 1, Appendix 2, and Appendix 3 for details regarding timing for assessments in the MAD phase.

6.12. Pharmacokinetic Assessments

Venous blood samples for determination of concentrations of KNX100 and its metabolites in plasma will be collected at the times presented in Appendix 3.

6.12.1. Determination of Drug Concentration in Pharmacokinetic Samples

Samples for determination of plasma concentrations of KNX100 and its metabolites will be analyzed using an appropriate bioanalytical method. Full details of the analytical method will be described in a separate bioanalytical report. All samples that are within the known stability of the analytes of interest (i.e., KNX100 and its metabolites) at the time of receipt by the bioanalytical laboratory will be analyzed. In addition, the PK samples may be subjected to further analyses by the Sponsor to further investigate the presence and/or identity of additional drug metabolites.

Drug concentration and exposure will be monitored in individual subjects and will form the basis of dose escalation decisions and study stopping criteria as defined in Section 6.20.1.

6.13. Clinical Laboratory Tests

Subjects will be required to fast overnight prior to collection of Clinical Laboratory Samples.

All subjects undergo screening via a blood test to rule out human immunodeficiency virus (HIV), Hepatitis B and C.

Serum human chorionic gonadotrophin will be determined in WOCBP at screening. A urine pregnancy test will be conducted in WOCBP upon admission (Day -3).

Urine will be collected for drug screening as well as urinalysis. Urinalysis will be assessed by dipstick analysis. Microscopic examination will be undertaken if there is an abnormality warranting further investigation.

An alcohol breath test will be conducted at Screening to determine eligibility.

Blood samples will be collected for hematology and serum chemistry as well as coagulation parameters.

Subjects will be in a seated or supine position during blood collection. A list of the clinical laboratory tests is provided in Appendix 5. Please refer to the SOA in Appendix 1 and Appendix 2 for details regarding timing for assessments.

6.14. Sample Collection, Storage, and Shipping

The site shall obtain, process, and freeze blood samples (at temperatures as per study manual) for the listed tests in preparation for shipment to a bioanalytical laboratory for analysis. Full details pertaining to the collection, handling, and shipment of these samples are provided in the laboratory manual. Safety laboratory assessments will be performed locally at the study site's laboratory, based on its established methods.

6.14.1. Handling, Storage, and Destruction of Biological Samples

After analysis, samples will be disposed of or retained for further use as described below. Any PK sample remaining after analysis for KNX100 and its metabolites, may be used for exploratory biomarker analyses. These analyses are for Sponsor use only and will not be included in the clinical study report.

Any biological samples collected for future research will be retained by the Sponsor or designee for a maximum of 15 years following the finalization of the clinical study report. The results from future analysis will not be reported in the clinical study report.

6.14.2. PK Samples

Incurred sample reproducibility analysis, if any, will be performed alongside the bioanalysis of the test samples and reported in a separate bioanalytical report. Samples for metabolite identification and/or analysis will be performed as an exploratory endpoint in this study. The results from the investigation(s) may not be reported in the clinical study report but will be reported separately in a PK report.

6.14.2.1. Timing of PK samples

Refer to Appendix 3 for details on the timing of PK samples. If more than one assessment is foreseen for a timepoint, assessments should be staggered in the same order throughout the study with the PK sampling point closest to the scheduled timepoint.

6.14.3. Collection of Plasma for Exploratory Analysis of PD Biomarkers

All subjects may also be asked to provide plasma samples which may be used to further investigate the relationship between PK and blood-borne biomarkers (yet to be identified).

Please refer to the SOA in Appendix 1 and Appendix 2 for details regarding timing for assessments.

6.15. End of Study Visit/Early Termination Visit

The End of Study (EOS)/Early Termination (ET) Visit will occur 7 days after last dose (±2 days) for Part 1 (SAD) and 7 days after the last dose (±2 days) for the MAD part of the study. This visit can also take place if a subject withdraws early from the study.

A serum pregnancy test will be performed for WOCBP. Hematology and coagulation, biochemistry, and urinalysis will be performed. The subject will be asked about AEs and prior and concomitant medications and an ECG will be conducted. Every attempt will be made for collection of data from withdrawn subjects via attendance to an EOS/ET Visit.

6.16. Dispensing Study Drug

The site pharmacy (or equivalent) associated with the study site is responsible for dispensing the appropriate randomized/blinded study product to site staff for dosing onsite. Dose escalation will progress upon CRC approval (refer to Section 9.8).

6.17. Adverse Events Assessments

Any new event or experience that was not present at Screening, or the worsening of an event present at Screening, is considered an AE (Section 6.17.1). The investigator is responsible for following all AEs from onset to adequate resolution (event is resolved or subject is medically stable), where possible (Section 6.17.8).

AEs will be documented from physical or neurological examination findings, clinically significant laboratory results (as per reference ranges indicated in the Study Procedures and Laboratory Manuals), vital signs, ECGs, EEGs, or any other assessments that are relevant to subject safety.

The PI is responsible for reporting of all safety events that occur within the AE reporting period and will classify severity (Section 6.17.3) and relationship to study drug (Section 6.17.4) for each event. Any event that meets the definition of a serious adverse event (SAE; Section 6.17.10) will be recorded and reported as an SAE.

The PI or designee is responsible for recording all AEs on the appropriate study specific CRF, regardless of the AE's likely relationship to study treatment. Any changes in severity of an AE will be recorded and any AEs characterized as intermittent will be documented for each episode.

The CRF has been designed to capture the data elements recommended for reporting of drug safety events under current International Conference on Harmonisation (ICH) guidelines (ICH E2A, E6).

6.17.1. Definition

This study will follow the definition of "adverse events" as specified in ICH E2A:

> "*Any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment."*

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE **does not** include:

- Pre-existing diseases or conditions present or detected at screening/baseline that do not worsen in severity or frequency.
- Hospitalization for evaluation or treatment of a condition that predated the initial dose of blinded study product and has not worsened in severity since that initial dose.
- Situations where an untoward medical occurrence has not occurred.
    - e.g., hospitalization for elective surgery, social and/or convenience admissions.
- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion)
    - Of note however, the condition that leads to the procedure *is* an AE, unless the condition pre-dated the initial dose of blinded study product and has not worsened in severity since that initial dose.
- Overdose of either study drug or concomitant medication without any signs or symptoms.

All AEs are classified as serious or non-serious. Refer to Section 6.17.10 for definition of an SAE.

6.17.2. Timing and Reporting Period

Treatment-emergent adverse events (TEAEs) are defined as any AE starting during the active treatment period (from first dose to 24 hours after the last dose of study treatment).

If an AE causes a subject to discontinue the study, the PI remains responsible for following the AE until the event is resolved or explained. Frequency of follow-up evaluation is left to the discretion of the PI.

In addition, any known untoward event that occurs subsequent to the AE-reporting period that the PI assesses as related to the study drug should also be reported as an AE.

6.17.3. Severity

The terms "severe" and "severity" are often used to describe the intensity of a specific AE; the AE itself, however, may be of relatively minor medical significance (such as severe headache). "Severe" is not the same as "serious", which is based on subject/event outcome or action criteria usually associated with events that pose a threat to a subject's life or functioning.

Seriousness (not severity) serves as a guide for defining regulatory reporting obligations (for communication of urgent safety concerns). Severity, however, is a useful parameter for defining the safety profile of a product at a more-granular level of detail that will later be helpful to support a broader range of medical decision making.

The investigator is required to grade the severity or toxicity of each AE and must assess the severity of AEs according to the Common Terminology Criteria for Adverse Events (CTCAE) scale, as follows:

- **Grade 1 Mild**; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- **Grade 2 Moderate**; minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental activities of daily living (ADL).
- **Grade 3 Severe** or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- **Grade 4 Life-threatening** consequences; urgent intervention indicated.
- **Grade 5 Death** related to AE.

6.17.4. Relationship/Causality

The investigator must record his/her opinion concerning the relationship of the AE to study therapy or other study intervention on the eCRF, using the categories provided therein. Investigators will determine relatedness of an event to study drug based on a temporal relationship as well as if the event is unanticipated or unexplained given the subject's clinical course, previous medical conditions, and concomitant medications.

- **Unrelated:** Not reasonably related to the investigational product. AE could not medically (pharmacologically/clinically) be attributed to the investigational product/study treatment under study in this clinical study protocol. A reasonable alternative explanation must be available.

- **Related:** Reasonably related to the investigational product. AE could medically (pharmacologically / clinically) be attributed to the investigational product/study treatment under study in this clinical study protocol.

6.17.5. Expectedness

Expected AEs related to KNX100 treatment are provided in the KNX100 Investigator's Brochure. Based on the observation of premonitory signs of seizure in dogs dosed at ≥25 mg/kg/day in preclinical studies, a potential for risk of seizure in humans following administration of KNX100 has been identified. This risk is mitigated by frequent monitoring of maximum plasma exposure and observation of EEGs both prior to and post dosing. Further information is provided in the Investigator's Brochure.

An unexpected AE is any AE that is inconsistent with the nature, severity, or frequency of AEs expected as related to KNX100 treatment.

6.17.6. Suspected Adverse Reaction

Suspected adverse reaction (SAR) means any AE for which there is a reasonable possibility that the drug caused the AE; that is, there is evidence to suggest a causal relationship between the drug and the AE. This definition is taken from US FDA regulation (21 CFR 312.32(a)) but is also consistent with the Australian NHMRC (2016) guidance *Safety Monitoring and Reporting in Clinical Trials Involving Therapeutic Goods*. Emergence of IED's recorded following dose administration will be considered an SAR.

6.17.7. Suspected Unexpected Serious Adverse Reaction

A suspected unexpected serious adverse reaction (SUSAR) is an AE that individually meets criteria for an SAE (Section 6.17.10) *AND* for an unexpected AE (Section 6.17.5) *AND* for a suspected adverse reaction (Section 6.17.6).

For this study and per 21 CFR 312.32, an unexpected SAE is a SUSAR only if there is evidence to suggest a causal relationship between the drug and the AE, such as:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome).
- One or more occurrences of an event that is not commonly associated with drug exposure but is otherwise uncommon in the population exposed to the drug (e.g., tendon rupture).
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates

those events occur more frequently in the drug treatment group than in a concurrent or historical control group.

6.17.8. Follow-up of Adverse Events

All measures required for AE management and the ultimate outcome of the AE must be recorded in the source document and reported to the Sponsor.

All AEs (both serious and non-serious) should be followed until the event is resolved or explained, even if the AE causes the subject to withdraw from the study and necessitates follow-up after study withdrawal. Frequency of follow-up evaluation, and method of follow-up (either directly or through an appropriate health care option) is left to the discretion of the investigator.

6.17.9. Clinical Laboratory and Investigational Findings Adverse Events

Abnormal laboratory findings and other abnormal investigational findings (for example, on an ECG trace) should not be reported as AEs unless they are associated with clinical signs and symptoms, lead to treatment discontinuation, or are considered otherwise medically important by the investigator. If a laboratory abnormality fulfills these criteria, the identified medical condition (i.e., anemia, increased alanine aminotransferase [ALT]) must be reported as the AE rather than the abnormal value itself.

The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with an underlying disease, unless judged by the investigator to be more severe than expected for the subject's condition.

All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 1 day after the last dose of study drug should be repeated until the values return to normal (or baseline) or are no longer considered clinically significant by the investigator or medical monitor. If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified, and the Sponsor notified.

All protocol-required laboratory assessments must be conducted in accordance with the laboratory manual and the SOA in Appendix 1 and Appendix 2. If laboratory values from non-protocol specified laboratory assessments performed at the institution’s local laboratory require a change in subject management or are considered clinically significant by the investigator (e.g., SAE, AE, or dose modification), then the results must be recorded in the CRF.

6.17.10. Serious Adverse Events

This study will follow the definition of serious adverse events as specified in ICH E2A:

> "*A serious adverse event (experience) or reaction is any untoward medical occurrence that at any dose:*
>
> - *results in death,*
> - *is life-threatening,*
>   - *NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.*
> - *requires inpatient hospitalization or prolongation of existing hospitalization,*
> - *results in persistent or significant disability/incapacity, or*
> - *is a congenital anomaly/birth defect,*
> - *other medically important serious medical event."*

In deciding whether an individual event meets the definition of an SAE, the following definitions will be used:

- Life threatening means that the subject was at immediate risk of death from the event as it occurred. It does not include events that may have caused death if they occurred in a more severe form.
- Inpatient hospitalization means a medical requirement for overnight admission to hospital, due to the severity of the event and the medical requirement for inpatient treatment and/or monitoring.
- Permanent disability means a permanent and substantial disruption of a subject's ability to carry out normal life functions.
- Important medical events will be considered an SAE when, based upon appropriate medical judgment, the AE may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

6.17.11. Reporting Adverse Events

Each AE is to be classified by the investigator as serious or non-serious (refer to Section 6.17.10). This classification determines the reporting procedures to be followed Table 6.

Table 6 Reporting Requirements for Adverse Events

Gravity	Reporting Time	Type of Report
Serious	Within 24 hours	SAE form
Non-serious	Per eCRF submission procedure	AE eCRF

Abbreviations: AE = adverse event; eCRF = electronic case report form; SAE = serious adverse event.

The investigator is responsible for reporting to the Sponsor within **24 hours** of becoming aware of an event that is an SAE. Local urgent safety measures (USMs) investigated by the site must also be reported to the Sponsor within **24 hours** of becoming aware of the event.

Local reporting requirements for events that meet the Australian definitions of significant safety issues (SSIs) (including USMs) are summarized in Appendix 6.

The Sponsor is responsible for assessing and categorizing the safety reports received from the investigator. Following receipt of the AE reports, the Sponsor or representative will code the events to standardized medical terminology using the Medical Dictionary for Regulatory Activities (MedDRA). During the study, all AEs will be coded to the MedDRA version that is current at the initiation visit of the first subject in the study.

The Sponsor is also responsible for communicating any important new information from investigators to the HREC and regulatory agencies, as applicable. The Sponsor activities linked to review, oversight and reporting of AEs will be conducted in compliance with applicable ICH guidelines (ICH E2A-E2F, ICH E6) both for expedited reporting of important new safety information that may arise during the study, as well as final reporting of the overall safety profile of the product at the end of the study.

6.17.11.1. Reporting Serious or Unexpected Adverse Events

The investigator is responsible for promptly reporting all SAEs to the Sponsor. The investigator will complete an SAE form and submit to the Sponsor within 1 working day after the site becomes aware of the event, irrespective of the causality or expectedness of the SAE.

The investigator is also responsible for cooperating with any Sponsor requests for further information about the SAE and responding to such requests in a timely manner to facilitate the Sponsor's fulfillment of regulatory reporting requirements. For example, typically SAE reports may require information such as a detailed description of events and investigator assessment of causality, copies of hospital reports, autopsy reports, or other relevant documents.

The Sponsor is responsible for expedited reporting of SUSARs to regulatory agencies. Expedited reporting of SUSARs will be performed in compliance with the local regulatory agency's requirements.

6.17.12. Expedited Reporting Requirements

Investigators are required to report any USMs to the Sponsor or its designee within 24 hours. The investigator will notify the HREC of SAEs and USMs, in accordance with HREC requirements and local laws and regulations. A copy of this notification must be provided to the Sponsor or its designee.

SAEs that are unexpected and considered related to the administration of the study drug will be reported by the Sponsor (or designee) to the appropriate regulatory authorities and other investigators in the form of an expedited safety report within 15 calendar days after receiving information on the SAE. The investigators will notify their reviewing HREC, and other committee(s) as required by institutional policies.

Fatal and life-threatening SUSARs will be submitted no later than 7-calendar days of first knowledge of the event and follow-up information submitted within an additional eight (8) days. The Sponsor will report any unexpected life threatening or fatal SAEs that are considered related to the investigational product to the appropriate regulatory authorities within 7 days of receiving the information.

6.17.13. Pregnancy

Pregnancies in subjects or partners must be reported to Sponsor or its designee within 24 hours of knowledge of the event up until and including the EOS visit. A subject who becomes pregnant will immediately be discontinued from study drug.

The pregnancy of a subject will be monitored for the full duration and/or followed until the outcome of the pregnancy is known.

In the event of pregnancy in the partner of a subject, the investigator should make every reasonable effort to obtain the female partner’s consent for release of protected health information and notify the Sponsor or its designee within 24 hours of knowledge of the event.

6.18. Safety Contact Information

Contact information for AE reporting may be found in a separate Study Reference Manual and Study Safety Plan. Site personnel may also contact the medical monitor for any safety concerns or questions.

6.19. Definition of Clinical Significance

A change in a subject's clinical status is regarded as important, whether or not it is due to an intervention in the context of a clinical study. The assessment of whether a result is clinically significant or not will be made, taking into account: a) whether the result is out of expected

values documented by laboratory reference ranges, and this is true after the assessment has been repeated, b) other symptoms being exhibited by the study subject and medical history and c) the opinion of the treating physician.

6.20. Study Stopping Rules

CTCAE, version V5, 27Nov2017 criteria will be used during this study (Refer also to Section 6.17.3).

The study will be discontinued for any of the following:

- Using CTCAE criteria, the study will be stopped for review if drug-related toxicities of Grade 3 or greater (or a SAE) are observed in 2 or more subjects on active treatment in that dose group.
- If any unacceptable safety findings are identified at any time during the study and/or if ≥1 subject experience a related SAE or a related significant AE that, in the opinion of the CRC, warrants discontinuation of the study.
- Any other safety concern based on the judgment of the PI and/or medical monitor and reviewed by the CRC.

If post dosing EEG's demonstrate evidence of epileptiform abnormalities, in any subject as evaluated by an epileptologist or a Level 3 ANZAN certified neurologist, all dosing will be suspended pending review and approval by the CRC.

The decision to stop the study will be made by the CRC. Refer to Section 9.8 for additional information on the CRC.

6.20.1. Maximum Clinical Exposure

Clinical PK will be considered as criteria for escalation of clinical doses. Dosing will not exceed $1/10^{th}$ of the exposure associated with the NOEL of 2.75 mg/kg observed in the 14-day dog EEG study (KNX100 C_{max} and AUC_{0-last} of 84.9 ng/mL and 82.7 hr × ng/mL, respectively and KNX100A C_{max} and AUC_{0-last} of 26.5 ng/mL and 45.7 hr × ng/mL, respectively).

6.20.2. Maximum Tolerated Dose Definition

The maximum tolerated dose (MTD) is defined as the dose below a non-tolerated dose and therefore can only be determined by the observation of a dose level, which is no longer considered acceptable in healthy subjects.

In this study, a dose will be considered non-tolerated and dose escalation will cease if 2 or more subjects in a cohort experience an AE of Grade 3 or higher occurring within 48 hours of administration of KNX100 or placebo at the dose level. Once the non-tolerated dose is defined,

the MTD will be confirmed at the previous dose level below the non-tolerated dose, or a dose between the non-tolerated dose and the last tolerated dose may be investigated. If the dose-escalation scheme is successfully completed for all escalating doses, 35 mg/day will be the highest dose studied (refer to Section 5.3.2 and Section 5.3.3 for the dosing schedule).

6.21. Removal of Subjects from the Study or Study Drug

6.21.1. Criteria for Discontinuation or Withdrawal of a Subject

The primary reason for discontinuation or withdrawal of the subject from the study should be recorded in the eCRF, using the following categories:

- Voluntary withdrawal by subject decision
  - The subject is at any time free to withdraw his or her participation in the study, without prejudice and independent of any decision concerning participation in other aspects of this study.
- AE
  - The subject has experienced an AE that requires early termination because continued participation imposes an unacceptable risk to the subject's health, or the subject is unwilling to continue because of the AE.
- Vital signs
  - Measurement of abnormal and out of range parameters on repeat analysis as required (postdose):
    - O_2 saturation of <90% for 60 seconds or longer
    - Respiratory rate of <8 breaths/minute for 2 or more minutes
    - Heart rate of <50 bpm
    - Systolic blood pressure of ≤80 mmHg
    - Temperature of ≤32°C
- EEG
  - Evidence of the emergence of epileptiform discharges as evaluated by an epileptologist or a Level 3 ANZAN certified neurologist.
- ECG
  - QTcF duration >475 ms and increase exceeding 40 ms in males
  - QTcF duration >475 ms and increase exceeding 60 ms in females

- Thyroid function tests outside the normal reference ranges (and upon repeat) and CTCAE Grade 2 or above. That is, symptomatic and requiring treatment.
- Laboratory Parameters
  - Safety laboratory tests that are outside the normal reference ranges (and upon repeat) and deemed clinically significant by the study PI.

- Pretreatment event
    - The subject has a pretreatment event that requires early termination because continued participation imposes an unacceptable risk, or the subject is unwilling to continue due to the pretreatment event.
- Pregnancy
    - If the subject is found to be pregnant, the subject must be withdrawn immediately.
- Major protocol deviation as judged by the investigator and/or Kinoxis.
    - The discovery post-randomization that the subject was incorrectly initiated on investigational drug, failed to meet protocol entry criteria, or did not adhere to protocol requirements **and** continued participation poses an unacceptable risk to the subject's health.
- Compliance
    - The subject has poor compliance with study drug or study procedures.
- Additional treatment
    - The subject requires treatment with another drug that will interfere with evaluation of the study drug.

Subjects that are withdrawn from the study but are evaluable will not be replaced. Any subject that is withdrawn and is not evaluable will be replaced to ensure a minimum number of evaluable subjects.

6.21.2. Subject Withdrawal of Consent

Subjects will be able to withdraw consent for study treatment, or discontinue from all study activities, at any time by their own volition with no impact on any future care they may require at the institution where the study is conducted.

Discontinuing study treatment does not withdraw consent for study follow-up. Subjects who wish to discontinue study treatment should continue to be followed unless they specifically also withdraw consent for continued follow-up.

Note that initial consent to participate in the study also irrevocably grants the Sponsor access to any data or specimens collected from the subject during the study. The subject may withdraw consent to participate at any time, but any data and research specimens collected prior to that withdrawal of consent remain in the study and remain the property of the Sponsor.

6.21.3. Study Withdrawal - Early Termination Procedures

Subjects who discontinue study treatment will have their EOS period planned, and safety follow up until that visit is completed (refer to the SOA in Appendix 1 and Appendix 2).

6.21.4. Lost to Follow-up

Subjects who do not present for study visits may be considered lost to follow-up only after the site has exercised and documented appropriate due diligence to attempt contacting the subject.

Prior to assessing a subject as lost to follow-up, the site must document at least 3 contact attempts on 3 separate days (using contact information and preferred mechanism as previously indicated by the subject), followed by a certified letter to the subject's last known address.

6.22. Discontinuation of the Study

The study will be discontinued if the Sponsor judges it necessary for any reason, including Sponsor acceptance of a CRC recommendation to stop the study for safety.

6.23. Appropriateness of Measurements

All procedures and assessments used to evaluate the safety and tolerability of study drug are widely used and generally regarded as reliable, accurate, and relevant. The specific safety monitoring, including clinical laboratory assessments, ECGs, and EEGs, are commonly used to assess healthy volunteers participating in a first-in-human study.

7. QUALITY CONTROL AND ASSURANCE

A study startup/investigator training will be held for all sites prior to first subject enrollment. To ensure accurate, complete, and reliable data, the Sponsor or its representatives will do the following:

- Provide instructional material to the study sites, as appropriate.
- Conduct a startup training session to instruct the investigators and study coordinators. This session will give instruction on the protocol, the completion of the eCRF, and study procedures.
- Make periodic visits to the study site.
- Be available for consultation and stay in contact with the study site personnel by appropriate communication media.
- Review and evaluate eCRF data and use standard computer checks to detect probable errors in data collection.
- Conduct a quality review of the database.

In addition, the Sponsor or its representatives will periodically check a sample of subject data recorded against source documents at the study site (refer to Section 9.6). The study may be audited by the Sponsor or its representatives, and/or regulatory agencies at any time. Investigators will be given notice before an audit occurs.

To ensure the safety of subjects in the study, and to ensure accurate, complete, and reliable data, the investigator will keep records of laboratory tests, clinical notes, and subject medical records in the subject files as original source documents for the study. If requested, the investigator will provide the Sponsor, applicable regulatory agencies, and applicable ethical review boards with direct access to original source documents (refer to Section 9.10).

8. PLANNED STATISTICAL METHODS

8.1. General Considerations

Kinoxis, or an appropriately qualified delegate, will be responsible for statistical analysis of all study data for the final clinical study report. The final analysis will be performed after all subjects have completed the EOS visit or discontinued the study. Other than analyses for CRC cohort reviews, no interim analysis is planned for this study.

Data will be summarized descriptively by study medication (placebo and treatment cohort). In addition, select safety and PK summaries may include pooled summaries with all subjects receiving a single dose. The descriptive summaries for the categorical variables will include counts and percentages. The descriptive summaries for the continuous variables will include means, medians, standard deviations, 25th and 75th quartiles, and minimum and maximum values. Unless specified otherwise, all data will be listed for all randomized subjects.

All confidence intervals will be 95%, unless stated otherwise.

Further details of definitions will be provided in a statistical analysis plan.

8.2. Determination of Sample Size

This study is descriptive in nature, and no formal comparisons or hypothesis testing will be performed between study medications or dosing levels. As such, no prospective calculations of statistical power have been made. The sample size has been selected to provide sufficient information to have preliminary characterization of safety, tolerability, PK and PD of the study drug.

8.3. Analysis Populations

The Safety Set (SAF) will include all subjects who received at least one dose of study treatment. Subjects will be analyzed according to the treatment received. The SAF will be used for all safety summaries.

A Per Protocol Set (PP) will include all subjects who received at least one dose of any amount of KNX100 and do not have major protocol violations. Major protocol violations are defined in

Section 9.9. A final determination of inclusion in the PP will be made by a blinded team prior to database lock and unblinding. The PP set may be used on select biomarker endpoints. Analyses conducted with the PP will be based on the assigned study medication.

For the PK assessment, the set of subjects will include those subjects who received at least one of study drug and had sufficient plasma KNX100 concentration data for calculation of PK parameters.

8.4. Demographics and Baseline Characteristics

Summary statistics will be provided for demographic and baseline characteristics in each analysis population and by cohort. No formal statistical comparisons will be performed. Demographic and baseline characteristic data for each subject will be provided in data listings.

8.5. Study Endpoints

8.5.1. Primary Safety Endpoints

The primary safety endpoints include:

- Incidence of TEAEs and TESAEs, related AEs, AEs leading to discontinuation, and AEs by severity.
- Clinical laboratory testing from protocol-specified standard urine and blood tests, including liver function tests (LFTs) and thyroid function tests (TFTs: triiodothyronine [T3], thyroxine [T4], thyroid stimulating hormone [TSH]).
- Change in clinical observations from baseline, including body temperatures, vital signs, ECGs, EEGs.

8.5.2. Secondary Pharmacokinetic Endpoints

The secondary PK endpoints include:

- Maximum observed concentrations (C_{max}) following dose administration.
- Time of C_{max} (T_{max}) following dose administration.
- Area under the concentration-time curve (AUC) from time zero to last quantifiable concentration ($AUC_{0\text{-last}}$), from time zero to 24 hours postdose administration ($AUC_{0\text{-}24}$), and, as data permit, from time zero to extrapolated to infinity ($AUC_{0\text{-}\infty}$).
- As data permit, additional disposition parameters including terminal half-life ($t_{1/2}$), volume of distribution (Vz/F), and oral clearance (CL/F) may be calculated.
- Accumulation ratio (RAC) for C_{max} and $AUC_{0\text{-}24}$; Day 7 vs Day 1 (MAD phase only).
- Other PK parameters will be calculated as deemed appropriate and as referred to in Section 8.7.

8.6. Statistical Analysis of Safety Variables

Safety endpoints for this study are provided in Section 8.5.1.

Summaries of safety data will be performed using the SAF. AE data will be coded to system organ class and preferred term using MedDRA. The MedDRA version will be specified in the study-specific data management plan (DMP).

The number and percentage of subjects experiencing any TEAEs, overall and by system organ class and preferred term, will be tabulated. TEAEs will also be summarized by severity and relationship. Treatment-related events, and other events will be listed. Concomitant medications will be summarized using frequency and percentage of subjects. Results and changes from baseline in vital signs, ECG readings, EEG, and hematology and clinical chemistry parameters will be tabulated at each visit. Abnormal ECG and laboratory findings will also be summarized.

Individual subject listings of prior and concomitant medications, EEG, vital signs, ECG data, and laboratory measurements will be provided.

In addition, ECG parameters will be analyzed as follows (data listings):

- 450 ms <QTcF/ QTcB ≤ 480 ms480 ms <QTcF/ QTcB ≤500 ms
- 500 ms <QTcF/ QTcB
- 30 ms <QTcF/ QTcB change from baseline ≤60 ms
- 60 ms <QTcF/ QTcB change from baseline

8.7. Statistical Analysis of Pharmacokinetic Variables

PK endpoints for this study are provided in Section 8.5.2. PK concentrations and parameters will be listed and summarized by dose, treatment group, and measurement time using appropriate descriptive statistics. The actual sampling times will be used in the parameter calculations, and PK parameters will be derived using standard non-compartmental methods. Where possible, the following PK parameters of KNX100 and any measurable metabolites will be determined:

- Following the single-dose part of the study:
    - Maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), terminal rate constant (λ_z), terminal half-life ($t_{½}$), area under the plasma concentration-time curve from zero to 24 hours (AUC_{0-24}), from zero to the time of the last measurable concentration (AUC_{0-t}) and from zero to infinity ($AUC_{0-\infty}$), apparent plasma clearance (CL/F), apparent volume of distribution (Vz/F), mean residence time (MRT).
- Following the multiple-dose part of the study:

- Maximum plasma concentration (C_{max}), time to maximum C_{max} (T_{max}), minimum plasma concentration (C_{min}), area under the plasma concentration-time curve from zero to the end of the dosing interval (AUC_{0-24}) will be assessed on both Day 1 and Day 7. The apparent oral plasma clearance (CL/F), the apparent volume of distribution (Vz/F), the terminal $t_{1/2}$, and the AUC extrapolated to infinity will be calculated on Day 7 (and Day 1 if data permit). The extent of accumulation following multiple dosing (accumulation ratio [RAC]) will be evaluated for C_{max} and AUC_{0-24}.

The C_{max} and T_{max} parameters will be determined through the concentration-time profiles. Where possible, the λ_z will be calculated by log-linear regression of the terminal portion of the concentration-time profiles where there are sufficient data and the $t_{½}$ will be calculated as ln 2/λz. The AUC_{0-t} and the AUC_{0-24} will be calculated using the linear trapezoidal rule. Where appropriate, the AUC_{0-t} will be extrapolated to infinity using λ_z to obtain AUC_{INF}. The AUC will be calculated using the linear trapezoidal rule. CL/F will be determined as dose/AUC_{INF}. Vz/F will be determined as Dose / (AUC_{INF} * λ_z).

Where possible, the appropriate PK parameters will also be determined for the metabolite (s) of KNX100.

Concentrations below the limit of quantification (BLQ) will be treated as zero for descriptive statistics. Mean BLQ concentrations will be presented as BLQ, and the standard deviation (SD) and coefficient of variation (CV) will be reported as not applicable. Missing concentrations will be excluded from the calculations.

8.8. Interim Analysis

No interim analysis is planned for this study.

9. ADMINISTRATIVE CONSIDERATIONS

9.1. Investigators and Study Administrative Structure

This study is sponsored by Kinoxis Therapeutics Pty Ltd. (Kinoxis). Information related to participating sites and investigators, qualified physicians, clinical laboratories, members of the CRC, and other relevant administrative personnel will be kept in the Study Master File.

9.2. Human Research Ethics Committee Approval

The study protocol and informed consent, as well as any amendments (Section 9.14), will be reviewed by the responsible HREC in accordance with Good Clinical Practice (GCP) prior to initiation at a particular site. Any member of the HREC who is directly affiliated with this study as an investigator or as site personnel must abstain from the vote on this study.

The HREC will be asked to document the date of the meeting at which the favorable opinion or approval was given along with a list of the members and voting members present. The favorable opinion or approval must also clearly identify the study along with the study protocol and information statement and informed consent form versions reviewed. Where possible, copies of the meeting minutes should be obtained.

Documentation from the HREC will be filed in the Investigator Site File. A copy will be filed in the Sponsor Study Master File at Kinoxis or with a designated organization.

Relevant safety information will be submitted to the HREC during study execution in accordance with national regulations and requirements. In the event of an SAE, the investigator, or Sponsor where applicable, will notify the relevant HREC of any SAEs and safety reports according to local regulation requirements (refer to Section 6.17.11 for more information regarding reporting SAEs).

9.3. Ethical Conduct of the Study

The Sponsor will conduct the study in accordance with the ethical principles that have their origin in the Declaration of Helsinki, are consistent with the GCP guidelines of the ICH, and according to applicable regulatory requirements.

The investigator, head of the medical institution, or designee will promptly submit the protocol to applicable ethical review boards.

As indicated in Section 9.5, an identification code assigned for each subject will be used in lieu of the subject's name to protect the subject's identity when reporting all study-related data.

9.4. Subject Information and Consent

A copy of the HREC approved version of the consent form will be provided to the Sponsor. Subjects who are identified as potentially being suitable to participate in the study will be given an information statement and consent form as approved by the Sponsor and by the site's HREC. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation.

The original signed consent form must be maintained at the site and made available for inspection, as appropriate. Additional information about the subject information and consent process is provided in Section 6.1.

9.5. Subject and Study Confidentiality

Subjects will be identified by their assigned subject number in all written communications between the investigator and Sponsor or its designees. Subject confidentiality is strictly held in trust by the participating investigators, research staff, and the Sponsoring institution and their agents, at a minimum complying with applicable local regulation. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or data will be released to any unauthorized third party, without prior written approval of the Sponsor.

Authorized representatives of the Sponsor, the HREC and regulatory agencies may inspect all documents and records required to be maintained by the site investigator (refer to Section 9.6). All laboratory specimens, evaluation forms, reports and other records that leave the site will be identified only by the Subject Identification Number (SID) to maintain subject confidentiality.

All study-related information provided by the Sponsor or its designees to the investigator and not previously published (including, but not limited to, the active study agent identity, the IB, the study protocol, verbal and written communication, study data, assay methods and scientific data), will be considered confidential. In addition, all information developed during the conduct of the clinical investigation of the study agent is also considered confidential. Neither the investigator nor any of his/her employees or agents shall disclose or use this information for any purpose other than the performance of the clinical study. Such information shall remain the confidential and proprietary property of the Sponsor, and disclosure to others will be limited to other physicians who are conducting studies with the same active study agent, the HREC, and the applicable regulatory authorities except by prior written permission of the Sponsor or its agents. At such time that information becomes widely and publicly available through no fault of the investigator, the obligation of nondisclosure toward that particular information will cease.

9.6. Case Report Forms and Study Records

Study data will be recorded/filed in subject medical files (source data) and transcribed into an electronic data capture system/CRF for reporting. All required study data will be entered onto the Sponsor-provided eCRF. All information in the eCRFs must be supported by original data in the subject's medical records. All medical records, laboratory printouts, notes made by the physician, and other materials, such as x-rays, will be considered source data and must be available for inspection by the Sponsor, the Sponsor's delegates, or government representatives.

9.6.1. Data Collection

Appropriate training prior to study initiation at that site will be conducted to assist with making entries and corrections to data entered into an eCRF. The site investigator is responsible for maintaining adequate case histories of study subjects, including accurate eCRFs and source documentation (where the source documents are distinct from the CRFs). The site investigator is responsible for the completeness, accuracy, legibility, and timeliness of the data reported; however, the investigator may exercise this responsibility by delegating the actual data collection, recording, and checking activities to appropriately qualified staff under the investigator's supervision.

Information in the database will refer to each study subject by a unique SID. Study subjects will not be identified by name, although the study data will, of necessity, include other personally identifying information such as date of birth.

9.6.2. Source Data

The site investigator is responsible for maintaining adequate and accurate source documents of all observations and data generated during this study. Source documents are the original documents, data, and records from which the subject's eCRF data are obtained. These include, but are not limited to, hospital records, clinical and office charts, laboratory and pharmacy records, radiographs, and correspondence. eCRF entries may be considered source data if the eCRF is the site of the original data recording or as defined in the eCRF completion guidelines or study manuals.

9.7. Study Monitoring

The study will be regularly monitored by a Sponsor representative contract research organization (CRO) according to ICH-GCP standards to ensure quality of the data, preservation of the rights and safety of subjects, and to ensure that the study is being conducted according to the protocol and to ethical and relevant regulatory requirements. The Sponsor or designee will assure the selection of qualified investigators, appropriate program centers, and review protocol procedures with the investigators and associated personnel prior to the program and during periodic monitoring visits.

The Sponsor or a designee will review eCRFs for accuracy and completeness during on-site monitoring visits and after their return from the clinical site. Data entered into eCRFs will be monitored by monitors that are adequately trained on the system. To ensure accurate, complete, and reliable data, the investigator will keep records of laboratory tests, clinical notes, and subject medical records in the subject files as original source documents for the program. The investigators will be required to agree to access by the Sponsor/Sponsor representatives to study documentation, including subject medical files and test results, for monitoring and auditing

purposes, and also to inspections by Regulatory Authorities or quality assurance auditors as requested. Discrepancies will be resolved with the investigator as appropriate.

The Sponsor or its designee will monitor the program using any of the following methods:

- Telephone contacts,
- Site visits, and
- Review of original subject records, CRFs, drug accountability, storage, and general program documentation.

The Sponsor reserves the right to terminate the program if access to source documentation of work performed in this program is denied to the Sponsor or regulatory representatives.

All SAEs will be reviewed within time frames mandated by company procedures and local regulatory requirements. All deaths and SAE reports will be reviewed by the clinical team during study execution to assure completeness and accuracy.

9.8. Cohort Review Committee

An independent CRC will oversee the progress of this study and periodically review the accruing safety data. The CRC is intended to ensure that treatment does not pose undue risk to subjects.

The CRC will include an appropriate group of professional specialties, including at a minimum, the following core individuals:

- Blinded PI or delegate (delegation only when the PI is not available and must be a physician).
- Blinded medical monitor or delegate (must be a physician).
- Blinded neurologist with expertise in EEG analysis and interpretation.
- Blinded pharmacokineticist

The roles, responsibilities, constitution, and operations of the CRC (including its specific composition and schedule for its assessments) will be described in the CRC Plan, which will be reviewed and signed by each member before the first subject is randomized and treated.

9.8.1. Blinded Data Review

For Part A (SAD), the CRC will review all safety and PK data of the Sentinel subjects, collected up to the 30-hour postdose timepoint, to enable decision to enroll the rest of cohort. The CRC will also conduct a blinded, cohort-escalation review after the final subject in a cohort has been dosed, using safety data from all subjects through at least the 30-hour-postdose visit, as well as

all available blinded PK and safety data from the current or previous cohorts. Upon acceptance of reviewed data, dosing for the 2 sentinel subjects in the next cohort may occur.

For Part B (MAD), the CRC will review all safety and PK data from each cohort collected up to and including the 24-hour post morning dose timepoint on Day 8. Additionally, the frequency and severity of the following events and findings will be used to determine the acceptability of escalation to the next cohort:

- Serious AEs
- Discontinuation of participation due to an AE
- Adverse events
- Clinically significant laboratory, EEG and ECG findings.
- Study medication sensitivities and reactions.

The safety observation period may be extended if additional safety data are required for any cohort. Based on all available data, a recommendation for dose escalation or changes to the protocol will be made in collaboration with the investigator.

Updated dispensing guidelines for each dose escalation will be provided based on all available data. Sponsor representatives will document cohort-escalation decisions in central files.

9.9. Protocol Violations/Deviations

This protocol is intended as a FIH study to establish the safety, tolerability, and PK characteristics of KNX100. As such, it is especially important that study sites and investigators conduct the study in accordance with the written protocol, to permit consistent data quality throughout the study.

Every protocol deviation (i.e., any change, divergence, or departure from the study design or procedures defined in the protocol) must be recorded in the subject record (source document) and on the CRF and must be reported to the PI. Protocol deviations will be assessed for significance by the PI, but also will be subject to review by the Sponsor.

Protocol violations (also known as “important protocol deviations”) are a subset of protocol deviations that may significantly impact the completeness, accuracy, and/or reliability of the study data, or that may significantly affect a subject's rights, safety, or well-being. At a minimum, these will include any instances of the following:

- Enrollment of a subject who did not satisfy the entry criteria.
- Failure to withdraw a subject from the study after the subject met withdrawal criteria.
- Subject received the wrong treatment or incorrect dose.

- Subject received an excluded concomitant treatment.
- Failing to collect data necessary to interpret primary endpoints.

The investigator is responsible for promptly notifying the Sponsor or Sponsor representative of any important protocol deviations/protocol violations. For these important deviations, the investigator and Sponsor will determine whether additional reporting is appropriate, considering any local requirements that exceed ICH recommendations.

9.10. Access to Source Documentation

The investigator is responsible for secure storage of completed source documentation and eCRFs until authorized to transfer the documentation to the Sponsor, or to dispose of the documentation. Please refer to Section 9.6.2 for a definition of source documentation.

Study source documentation and CRFs are subject to inspection by the Sponsor, its representatives, the HREC, and Regulatory Authorities, at times of their choosing. The study site must allow study-related monitoring audits and/or inspections when so requested by these authorized persons. Please refer to Section 9.7 for more information on site monitoring audits and inspections.

9.11. Data Generation and Analysis

The Sponsor is responsible for designating appropriate personnel to build and maintain a clinical database appropriate for a pivotal study.

All data from the CRFs will be transferred into a computer database, which will be designed in compliance with applicable conventions for standardized data sets, and with appropriate range checks. Appropriate Sponsor personnel/designees will check the database against the CRF for accuracy. Sponsor personnel/designees will also work with staff at study sites for clarification of data entries as needed (refer to Section 9.7).

9.12. Retention of Data

Both investigators and the Sponsor are responsible for secure storage of completed CRFs during the conduct of the study.

The Sponsor is responsible for generating backup copies of the CRFs and database and is responsible for securely storing the backups separately from the primary files.

Access to the database will be restricted by password. Computers will be password protected and held in secure monitored facilities.

All data derived from the study will remain the property of the Sponsor. Records must be retained in accordance with the current ICH Guidelines on GCP. All essential study documents including records of subjects, source documents, eCRFs and study drug inventory must be kept in a study specific file.

The site investigator will not dispose of any records relevant to this study without the written permission from the Sponsor and will provide the Sponsor the opportunity to collect such records. The site investigator will notify the Sponsor in writing of their intent to destroy all such material. The Sponsor will have 30 days to respond to the site investigator's notice, and the Sponsor shall have a further opportunity to retain such materials at the Sponsor's expense.

If a site investigator moves, withdraws from an investigation, or retires, the responsibilities for maintaining the records may be transferred to another person who will accept responsibility. Notice of transfer must be made and agreed by the Sponsor.

Per ICH GCP (R2), (ICH Guideline E6), at a minimum the Sponsor-specific essential documents (including subject-specific source documents) should be retained until either of the following is true:

- At least 2 years after the last approval of a marketing application in an ICH region (and until there are no further marketing applications contemplated in ICH regions),
  or
- At least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product.

However, source data and other essential documents should be retained for a longer period if required by local regulations.

In Australia, records must be kept for at least 15 years after the completion of a clinical study.

9.13. Financial Disclosure

The investigators and the Sponsor will sign a clinical study agreement prior to the start of the study, outlining the overall Sponsor and investigator responsibilities in relation to the study.

All investigators and the Sponsor will declare any conflicts of interest. If potential conflicts of interest are present, the Sponsor and investigator are jointly responsible for determining appropriate controls to mitigate risks to data integrity at the investigator's site.

Financial Disclosure Statements are required for all investigators on this study worldwide, as required by FDA regulation 21 CFR 54.

9.14. Changes to the Clinical Study Protocol

Changes to the clinical study protocol will be documented in writing. Substantive amendments will usually require submission to the IND and to the relevant HREC for approval or favorable opinion. In such cases, the amendment will be implemented only after approval or favorable opinion has been obtained (refer to Section 9.2).

Minor (non-substantial) protocol amendments, including administrative changes, will be filed by the Sponsor and at the site. They will be submitted to the relevant HREC or to regulatory bodies only where requested by pertinent regulations. Any amendment that could affect the subject's agreement to participate in the study requires additional informed consent prior to implementation, following the process as described in Section 6.1 and Section 9.4.

9.15. Disruption Due to Coronavirus Disease 2019 (COVID-19) Pandemic

If a clinical site or investigator experiences disruption due to COVID-19 (or other natural disaster), a shortened list of assessments may be implemented as an immediate safety measure to ensure at least safety, primary, and key secondary endpoints are captured for any dosed subject. Assessments may be performed by telephone if possible.

If a subject develops COVID-19, this will be reported and followed as an AE per Section 6.17. The investigator will follow the standard operating procedures for the site as well as local, regional, and national guidelines for COVID-19. Investigators should use clinical judgement as to whether a subject should continue, temporarily stop, or permanently withdraw from the study.

9.16. Publication and Data Disclosure Policy

By signing the study protocol, the investigator agrees with the use of the results of the study for the purposes of national and international registration, publication, and information for medical and pharmaceutical professionals. Regulatory Authorities will be notified of the investigator's name, address, qualifications, and extent of involvement.

All data generated from this program are the property of the Sponsor and shall be held in strict confidence along with all information furnished by the Sponsor. Independent analyses and/or publication of these data by the investigator or any member of his/her staff are not permitted without prior written consent of the Sponsor. An investigator may not publish any data (poster, abstract, paper, etc.) without having consulted with the PI and Sponsor in advance and having received a written approval for such a publication.

Further details on the publication process are provided in individual contractual agreements signed by the investigators and the Sponsor.

10. REFERENCE LIST

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Appendix 1 Schedule of Assessments - SAD

	Screening	**Treatment**			**Follow Up/ ET/EOS[1]**
Study Day	-28 to -2	-1	1	2 [2]	7 (±2)
Informed Consent	X				
Inclusion/Exclusion Criteria[3]	X	X			
Randomization[4]		X			
Height and BMI	X				
Medical History and Demographics	X				
Physical and Neurological Exam[5]	X	X	X	X	X
Vital Signs[6]	X	X	X	X	X
Laboratory Tests[7]					
Serology	X				
Hematology	X	X	X	X	X
Serum Chemistry	X	X	X	X	X
Urinalysis[8]	X	X	X	X	X
Coagulation	X	X	X	X	X
Pregnancy Test[9]	X				X
Drug of Abuse Screening[10]	X	X			
Alcohol Breath Test	X				
Study Drug Administration[11]			X		
PK Assessments[12]			X	X	
Biomarker Sample[13]		X	X		X
EEG[14]	X		X	X	
12-Lead ECG[15]	X	X	X	X	X
Adverse events	X	X	X	X	X

	Screening	**Treatment**			**Follow Up/ ET/EOS[1]**
Concomitant Medications	X	X[3]	X	X	X
Inpatient Component[16]	X	X	X	X	

Abbreviations: BMI = body mass index; ECG = electrocardiogram; EEG = electroencephalography; ET = early termination; EOS = end of study; PK = pharmacokinetics; SAD = single ascending dose.

[1] All subjects will be required to attend an ET/EOS visit, even if they have withdrawn or been removed from the study. For completed subjects, this will be at Day 7.

[2] For sentinel subjects this will include a follow up phone call at 48 hours.

[3] Confirmation of eligibility based on inclusion/exclusion criteria.

[4] Randomization to occur after confirmation of eligibility on Day -1 and prior to dosing on Day 1.

[5] An abbreviated neurological examination will be undertaken to include: light touch/power in limbs, brief cranial nerve examination and recording of any other abnormal movements; to occur on Day-1, Day 1 predose and at 30 minutes (± 15 minutes) postdose, and Day 2. Physical and neurological examination on Day 2 should be performed pre-discharge.

[6] Vital signs collection includes systolic and diastolic blood pressures, pulse rate and body temperature which will be measured with the subject to be seated or semi-supine after resting for at least 3 minutes. Vital signs also include respiratory rate, continuous pulse oximetry and saturated oxygen, which will be undertaken via telemetry within 2 hours predose and at 30 hours (± 30 minutes) after dosing. Measurements of all vital signs will be recorded within 1 hour predose, and at 30 minutes, 1-, 1.5-, 2-, 4-, 6-, 8- ,12-, 24- and 30-hours postdose (± 15 minutes). Peak flow measurement will also be undertaken within 1 hour predose as well as at 1-, 2-, and 4-hours (± 15 minutes) postdose. The highest of 3 peak flow measurements, at each timepoint, shall be recorded.

[7] Subjects should fast overnight prior to collection of laboratory tests at Screening. The Day 1 laboratory tests will be undertaken at 1 hour postdose (± 15 minutes) and at 24 hours (± 15 minutes) postdose (Day 2).

[8] The Day 1 urinalysis tests will be undertaken at 1-hour postdose (± 60 minutes) and at 24 hours (Day 2) (± 60 minutes) postdose.

[9] Serum pregnancy test at Screening and End of study visit. A urine pregnancy test will be conducted in women of child-bearing potential on admission to unit on Day -3.

[10] Test may be repeated if required.

[11] Each subject will receive study drug based on randomization (placebo or KNX100), after a 10 hour fast.

[12] Please refer to Appendix 3 for information related to PK assessments and sampling times.

[13] Plasma samples for biomarker analyses will be collected predose (Day -1), on Day 1 at 1-hour postdose (± 15 minutes), and at EOS/EOT.

[14] EEG: Please refer to Appendix 3 for information related to EEG assessments.

[15] Please refer to Appendix 3 for information related to ECG assessments which will be performed in triplicate. The timing and number of ECGs may be altered, depending on the emerging PK and safety profile. Additional ECGs should also be collected if a subject experiences a cardiac adverse event.

[16] Participants will be admitted to the Phase 1 unit on Day -3 so they can sleep for 4 hours prior to undertaking their sleep deprived EEG on Day -2, through to 30 hour testing on Day 2.

Appendix 2 Schedule of Assessments – MAD

	Screening	Treatment									ET/EOS[1]
Study Day	-28 to -2	-1	1	2	3	4	5	6	7	8 [2]	14 (± 2)
Informed Consent	X										
Inclusion/Exclusion criteria[3]	X	X									
Randomization[4]		X									
Height and BMI	X										
Physical and Neurological Exam[5]	X	X	X	X	X	X	X	X	X	X	X
Gastrointestinal Symptoms[6]	X	X	X	X	X	X	X	X	X	X	X
Vital Signs[7]	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X
Laboratory Tests[8]											
Serology	X										
Hematology	X	X	X	X		X		X	X	X	X
Serum Chemistry	X	X	X	X		X		X	X	X	X
Urinalysis	X	X	X	X		X		X	X	X	X
Coagulation	X	X	X	X		X		X	X	X	X
Pregnancy Test[9]	X	X									X
Drug Abuse Screening	X	X[10]									
Alcohol Breath Test	X	X[10]									
Study Drug Administration[11]			X	X	X	X	X	X	X		
PK Assessments[12]			X			X[12]			X		
Biomarker Sample[13]		X	X	X	X				X		X

	Screening	Treatment									ET/EOS[1]
Study Day	-28 to -2	-1	1	2	3	4	5	6	7	8 [2]	14 (± 2)
12-Lead ECG[14]	X	X	X	X	X	X	X	X	X		X
EEG[15]	X		X	X	X	X	X	X	X	X	
C-SSRS[16]	X					X			X		
Adverse Events	X	X	X	X	X	X	X	X	X	X	X
Inpatient component[17]	X	X	X	X	X	X	X	X	X	X	

Abbreviations: BMI = body mass index; C-SSRS = Columbia Suicide Severity Rating Scale; ECG = electrocardiogram; EEG = electroencephalography; EOS = end of study; ET = end of treatment; PK = pharmacokinetics; MAD = multiple ascending dose; SAD = single ascending dose.

[1] All subjects required to attend an ET/EOS visit, even if they have withdrawn or been removed from the study.

[2] Day 8 is 24-hours (± 1 hour) after administration of the last morning dose. This visit will be used to collect the "24-hour" assessments.

[3] Confirmation of eligibility based on inclusion/exclusion criteria.

[4] Randomization to occur after confirmation of eligibility on Day -1 and prior to the first dose on Day 1.

[5] An abbreviated neurological examination will be undertaken to include: light touch/power in limbs, brief cranial nerve examination up to 1 hour prior to the first dose and at 30 minutes (± 15 minutes) post morning dose.

[6] An assessment of bowel movements including any incidence of constipation or changes in bowel habits will be conducted daily. On Day -1 and Day 1, the gastrointestinal screening assessments will be conducted as part of physical examinations. On Days 2, 3, 4, 5, 6, 7, 8 and Day 14, a gastrointestinal questionnaire will be administered within 30 minutes prior to morning dosing.

[7] Vital signs will be measured with the subject to be seated or semi-supine after resting for at least 3 minutes. Measurements will include systolic and diastolic blood pressures, pulse rate, body temperature, respiratory rate, saturated oxygen and pulse oximetry which will be recorded via telemetry within 2 hours predose and at 2 hours postdose (± 15 minutes). Peak flow measurement will also be undertaken within 1 hour predose as well as at 1-, 2-, and 4-hours (± 15 minutes) postdose. The highest of 3 peak flow measurements, at each timepoint, shall be recorded. For MAD Cohort 3, vital signs, telemetry, and peak flow measurements will be recorded following each dose as per the schedule above.

[8] Laboratory tests will be undertaken as depicted above. The Day 1 laboratory tests will be undertaken 1 hour post morning dose (± 15 minutes).

[9] A serum pregnancy test will be undertaken at screening and End of Study visit. A urine pregnancy test will be conducted in women of child-bearing potential on admission (Day -3).

[10] Drug abuse screening and alcohol breath test will be conducted at admission (Day -3).

[11]Subjects in Cohorts 1 and 2 of the MAD study phase will be dosed daily for 7 days at approximately the same time each day. Each subject will receive study drug based on randomization (placebo or KNX100), after a 10 hour fast. Subjects will remain fasted until 2 hours (±15 minutes) post study drug administration.

Subjects in Cohort 3 of the MAD study phase will be dosed twice daily (b.i.d) with doses administered approximately 8 hours (± 30 minutes) apart. The first dose will be administered following a 10 hour overnight fast, and subjects will remain fasted until 2hours (±15 minutes) post study drug administration. Subjects will be required to fast 2 hours (± 15 minutes) prior to administration of the second dose of study drug and will remain fasted until 2hours (±15 minutes) post study drug administration.

[12]Please refer to Appendix 3 for information related to PK assessments and sampling times. The timing and number of PK samples may be altered depending on emerging data and safety profile. Please note, Day 4 predose sample is not required for Cohort 3.

[13] Plasma samples for biomarker analyses will be collected predose (Day -1), and 1 hour post morning dose (± 15 minutes) on Days 1, 2, 3, 7 and at the ET/EOS visit.

[14] Please refer to Appendix 3 for information related to ECG assessments. ECGs will be undertaken in triplicate. The timing and number of ECGs may be altered, depending on the emerging PK and safety profile. ECGs should also be collected if a subject experiences a cardiac adverse event.

[15] EEG: Please refer to Appendix 3 for information related to EEG assessments.

[16] C-SSRS: will be administered predose at screening and then within 4 hours post the morning dose on Days 4 and 7.

[17] Subjects will be admitted to the unit on Day -3 to undertake their sleep deprived EEG. Subjects will remain in the unit during the entire dosing phase and be discharged after collection of Day 8 assessments.

Appendix 3 PK Blood Sampling, EEG and ECG Schedule During Dosing Periods

Time Relative to Dose	Part A (SAD, HV)						Part B (MAD, HV)					
	Day 1			Day 2[1]			Day 1-7					
							Cohorts 1-2			Cohort 3		
	PK[2]	ECG[3]	EEG[4]	PK[2]	ECG[3]	EEG[4]	PK[2]	ECG[3]	EEG[4]	PK[5]	ECG[3]	EEG[4]
Predose	X	X	X	X		X	X	X	X	X	X	X
15 minutes	X						X					
30 minutes	X						X		X	X		X
1 hour	X	X					X	X		X	X	
2 hours	X	X					X			X		
4 hours	X	X					X			X		
8 hours	X	X					X			X		
8.5 hours										X		
9 hours										X		
10 hours										X		
12 hours	X						X			X		
16 hours	X	X								X		
24 hours		X	X	X		X			X			X
30 hours				X								

Abbreviations: ECG = electrocardiogram; EEG = electroencephalography; HV = healthy volunteer; MAD = multiple ascending dose; PK = pharmacokinetics; SAD = single ascending dose.

[1] For Part A (SAD), Day 2 is the 24-hour timepoint after last study drug administration. For Part B (MAD), Day 8 is the 24-hour timepoint after last study drug administration (Cohorts 1 and 2) and 24-hours post morning dose (Cohort 3). This visit will be used to collect only the "24-hour" samples.

[2] During Part A (SAD), PK samples and time windows are predose, and 15 minutes (± 2 minutes), 30 minutes (± 2 minutes), 1 hour (± 5 minutes), 2-, 4-, 8-, 12-, and 24- and 30-hours (± 30 minutes) postdose. During Part B (MAD Cohorts 1 and 2), PK samples will be taken at predose and 15 minutes (± 2 minutes), 30 minutes (± 2 minutes), 1-hour (± 5 minutes), 2-, 4-, 8-, and 12- hours (± 15 minutes) postdose on Days 1, , 4 and 7 The timing and number of PK samples may be altered depending on emerging data and safety profile.

[3] Part A (SAD) ECGs and time windows are Screening, Day -1, predose, 1 hour (± 2 minutes), 2-, 4-, 8-, and 16-hours (± 5 minutes), and 24-hours (± 30 minutes). For the MAD part of the study, ECGs will be undertaken at Screening, Day -1, predose, and 1-hour postdose (± 30 minutes) on Day 1 to Day 7. ECGs will be measured with the subject to be seated or semi-supine after resting for at least 3 minutes.

[4] A 1-hour (± 15 minutes) EEG will be collected during Part A (SAD) at screening under sleep deprived conditions (after only 4 hours of sleep) and 24 hour ambulatory EEGs will be collected from at least 30 minutes (± 10 minutes) predose until 24 hours (± 1 hour) postdose (Day 1). During Part B (MAD), a 1-hour (± 15 minutes) EEG will be collected at screening under sleep deprived conditions (after only 4 hours of sleep) and a 24-hour ambulatory EEG will be collected from at least 30 minutes (± 10 minutes) predose (Day 1) until at least 2 hours post morning dose (Day 2). This will incorporate both pre- and post-Day 2 dosing. On Days 3, 4, 5, and 6, a 2-hour (± 10 minutes) postdose EEG will be collected commencing at least 30 minutes predose until at least 120 minutes post morning dose. On Day 7, a 24-hour ambulatory EEG will be collected from at least 30 minutes (± 10 minutes) predose until 24 hours (± 1 hour) post morning dose (Day 8). Additional EEGs may be performed as deemed necessary and as data emerges from study cohorts.

[5] During Part B (MAD Cohort3), PK samples will be taken at predose and 30 minutes (± 2 minutes), 1-hour (± 5 minutes), 2-, 4-, 8- (predose for 2nd dose), 8.5-, 9-, 10-, 12- and 16- hours (± 15 minutes) postdose (Days 1 and 7 only). Note that the 8.5-, 9-, 10-,12 and 16-hour timepoints represent 0.5-, 1-, 2-, 4-, and 8 hours ***after*** the 2nd dose).

Appendix 4 KTX-101 Normal Ranges for Vital Signs and ECG Parameters

VITAL SIGNS

Parameter	Normal Range	Units
Pulse Rate	50 – 100	beats per minute (bpm)
Systolic Blood Pressure	90 - 140	mm Hg
Diastolic Blood Pressure	40 - 90	mm Hg
Respiratory Rate	10 - 22	respirations per minute
Oral Temperature	35.5 – 37.5	°C
Pulse Oximetry	≥ 95	%

ECG PARAMETERS

Parameter	Normal Range	Units
Heart Rate	50 – 100	bpm
PR Interval	≥ 120 to ≤ 220	msec
QRS Duration	< 120	msec
QT Interval	< 500	msec
QTcB	≤ 450 (both genders)	msec
QTcF	≤ 450 (both genders)	msec

Appendix 5 List of Laboratory Tests

Hematology:

- Hematocrit (Hct)
- Hemoglobin (Hgb)
- Mean corpuscular volume (MCV)
- Platelet count
- Red blood cell (RBC) count
- White blood cell (WBC) count with differential

Urinalysis:

- Bilirubin
- Glucose
- Ketones
- Leukocytes
- Nitrite
- Blood
- pH
- Protein
- Specific gravity
- Urobilinogen

If there are any abnormalities considered clinically significant, the urine sample will be sent for microscopic examination.

Serology:

HBsAg, HCV, and HIV

Serum Chemistry:

- Albumin (ALB)
- Alkaline phosphatase (ALP)
- Alanine aminotransferase (ALT; SGPT)
- Aspartate aminotransferase (AST; SGOT)
- Bicarbonate
- Blood urea nitrogen (BUN)
- Calcium (Ca)
- Chloride (Cl)
- Creatinine
- Gamma-glutamyl transferase (GGT)
- Glucose
- Lactate dehydrogenase (LDH)
- Phosphate
- Potassium (K)
- Sodium (Na)
- Thyroid stimulating hormone (TSH)
- Free triiodothyronine (T3)
- Free thyroxine (T4)
- Total bilirubin
- Total protein

Coagulation:

- Prothrombin time (PT)
- Activated partial thromboplastin time (PTT)

Serum human chorionic gonadotropin (hCG) (only for females who are not diagnosed as postmenopausal) Urine pregnancy test	Urine Drug Screen for benzodiazepines, cannabinoids, methadone, opiates, sympathomimetic amines, and cocaine. Panel Drug Screen for Amphetamines (AMP) Methamphetamines (MET), Methadone (MTD), Barbiturates (BAR), Benzodiazepines (BZO), Cocaine (COC), Opiates (OPI), 3,4-Methylenedioxymethamphetamine (MDMA), Phencyclidine (PCP), Tetrahydrocannabinol (THC)

Normal reference ranges will be included in the Study Procedures Manual and /or Laboratory Manual.

Appendix 6 Country-Specific Safety Reporting (Australia)

For clinical sites in Australia, the following local requirements apply, in addition to safety reporting requirements discussed in the main protocol.

Significant Safety Issue

In Australia, an SSI is defined as "a safety event that could adversely affect the safety of subjects or materially impact on the continued ethical acceptability or conduct of the trial" (Reference: NHMRC [2016] Guidance: Safety monitoring and reporting in clinical trials involving therapeutic goods).

Kinoxis is responsible for reporting to the Australian Therapeutic Goods Administration (TGA) in a timely fashion for any SSIs that occur at any site on the study worldwide.

Urgent Safety Measure

In Australia, USMs are one type of SSI where Sponsors or trial investigators act immediately to protect participants from an immediate hazard to their health and safety." (Reference: NHMRC (2016). Guidance: Safety monitoring and reporting in clinical trials involving therapeutic goods)

Kinoxis is responsible for reporting to the Australian TGA and the HREC (Human Research Ethics Committee) within 24 hours of learning of any USMs that occur at any site on the study worldwide.